Energy Benchmarking for Efficient, Low-Carbon Water Recycling Operations (RP2017)

Final Report

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The author(s) confirm(s) that this document has been reviewed and approved by the project's steering committee and by its program leader. These reviewers evaluated its:

- originality
- methodology
- rigour
- compliance with ethical guidelines
- conclusions against results
- conformity with the principles of the Australian Code for the Responsible Conduct of Research (NHMRC 2007), and provided constructive feedback which was considered and addressed by the author(s).

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Acronyms

AGS – aerobic granular sludge

BOD_5 – five-day biochemical oxygen demand

CAS – conventional activated sludge

COD – chemical oxygen demand

GHG – greenhouse gas

KPI – key performance indicator

kWh – kilowatt hours

MBR – membrane bioreactor

N_2O – nitrous oxide

PE – population equivalent

SBR – sequencing batch reactor

TN – total nitrogen

UV – ultraviolet

WSAA – The Water Services Association of Australia

WWTP – wastewater treatment plant

Executive Summary

Wastewater treatment plays a pivotal role in the protection of public and environmental health in urban precincts and in the recovery of scarce water and energy resources for an increasingly urban and growing global population. Yet wastewater treatment operations are among the most energy-intensive within urban precincts and so there is considerable scope to optimise wastewater treatment plants to improve their energy efficiency and reduce associated carbon emissions and broader environmental impacts. New and emerging wastewater treatment technologies offer the promise of improved treatment outcomes, but it is important for industry to fully understand the performance of these new technologies across a range of criteria before implementation.

This project has undertaken research into two core areas of relevance to the Australian water industry:

1) Energy efficiency in wastewater treatment via energy benchmarking methodology; and

2) Investigating the performance of aerobic granular sludge technology for wastewater treatment.

For the first research component, the project has produced a comprehensive, critical review of international energy benchmarking methodology in the water industry internationally. This review delivers for the first time a complete understanding of the development, evolution and application of European (predominantly German) energy benchmarking methods, unlocking a rich and valuable, but previously inaccessible, knowledge base for an international industry audience. The review gives detailed summaries of the key information and energy benchmarks required by water industry practitioners to enable them to perform with confidence their own WWTP energy assessment and optimisation activities to help achieve best practice WWTP energy efficiency. A comprehensive reference library resource for the water industry including resources relating to low energy/carbon wastewater treatment and water recycling operations has also been produced as a complement to this review.

Next the project utilised a national dataset of the energy performance of some 244 Australian wastewater treatment plants to develop a suite of new and updated WWTP energy benchmarks for industry to use in future energy benchmarking assessments and efficiency optimisations. While preliminary, these benchmarks represent a first step towards the development of Australian-specific energy benchmark key performance indicators for the local water industry. For the first time, electricity-related carbon emissions intensity performance data are also benchmarked for Australian wastewater treatment operations at both a state and national level.

Finally, the financial and environmental performance of two wastewater treatment systems was investigated using data gathered from full-scale Australian WWTPs as a case study. The operating costs of two contrasting disinfection technologies (ultraviolet light and chlorine) was compared, with chlorine some 10-fold lower cost that ultraviolet disinfection. The environmental performance of two contrasting wastewater treatment systems were also compared: state-of-the-art membrane bioreactor technology, compared to conventional activated sludge technology. Comprehensive data were gathered on both treatment systems relating to their construction and operation and performance assessed via environmental life cycle assessment. Preliminary results across the seven impact categories suggest lower environmental impacts from conventional activated sludge operations compared to the membrane bioreactor process, with the exception of the ozone depletion potential impact category. Information from this case study investigation provides the water industry with new insights into the economic and environmental performance of key wastewater treatment processes and systems for the sustainable planning and delivery of its future WWTP operations.

The second research component of the project involved pilot-scale research investigations into the performance of an emerging wastewater treatment technology – aerobic granular sludge. This technology is one of emerging interest to the Australian water industry, particularly for retrofitting of existing treatment operations for conversion to aerobic granular sludge; however, gaps in our understanding of this technology remain and so formed the basis for this component of project research. Research done to understand the role of wastewater feeding strategy (anaerobic or split anaerobic–aerobic) on aerobic granular sludge development and functional performance showed for the first time that a dedicated anaerobic feed is not universally required for successful aerobic granular sludge development and operation. New insights into the functional microbiology of aerobic granular sludge were also delivered in the context of high saline wastewater treatment. These findings will be of value to water industry members planning to retrofit existing conventional activated sludge-based processes to operate with aerobic granular sludge.

Next the capacity of aerobic granular sludge operations to treat and remove microbial pathogens was assessed. Such information on microbial pathogen removal performance during wastewater treatment is crucial for the water industry to know in order to be able to maintain adequate downstream treatment and disinfection for public health protection upon effluent discharge to receiving waterways, or during effluent reuse in water recycling schemes. Results confirmed for the first time that the adoption of aerobic granular sludge operation would not adversely impact water quality in such a way that could impact downstream tertiary disinfection processes or compromise public health protection barriers already in place for traditional conventional activated sludge-based systems.

Finally, the dynamics of direct emissions of the potent greenhouse gas nitrous oxide was assessed for aerobic granular sludge operations and compared side-by-side to conventional activated sludge-based operations. Results showed that when operated under operationally-relevant organic loading rates, nitrous oxide emissions were comparable between aerobic granular sludge and conventional activated sludge-based operations. Exceeding a loading rate of 0.6 kg chemical oxygen demand/m^3/d, however, resulted in higher emissions of nitrous oxide by aerobic granular sludge operations compared to conventional activated sludge. This aspect of the research is still ongoing, but once complete, results will help the water industry better understand the full environmental consequences of any future technological transition to aerobic granular sludge-based wastewater treatment processes.

1. Introduction

Wastewater treatment plays a pivotal role in the protection of public and environmental health in urban precincts and in the recovery of scarce water and energy resources for an increasingly urban and growing global population. With the progressive implementation of increasingly stringent human and environmental health regulations in recent decades, the water industry has seen a steady progression from simple low-cost wastewater treatment processes, to more advanced, highly engineered processes of increasing technological complexity and energy use intensity (Chang *et al.* 2008). This progressive intensification of energy demands for more advanced wastewater treatment has been brought sharply into focus in recent years by dramatic increases in the cost of energy, including electricity, as well as increasing volatility in energy tariffs (Escribano *et al.* 2011). At the same time, there has been an increased environmental awareness within the water sector (e.g. Lundie *et al*., 2008), including a focus on understanding and minimising greenhouse gas (GHG) emissions as water utilities pursue strategic objectives of carbon neutrality (Foley *et al.* 2010). In combination, these factors have increased the pressure on energy-hungry industries and facilities like wastewater treatment plants (WWTPs) to look for ways to minimise operational energy use and improve the overall sustainability of their operations. These considerations apply also to new and emerging wastewater treatment technologies and there is a need to better understand the technological and environmental performance of such technologies prior to industry adoption. As such, the overarching objective of this research project was to provide the water industry with new and improved information to facilitate more energy-efficient, cost-effective and environmentally-benign wastewater treatment operations into the future.

2. Changes to project scope

In consultation with water industry stakeholders, changes were made to the original project scope in order to better reflect the needs of the industry partners and also to capitalise on areas of expertise and research facilities available within the industry steering committee (see Table 1). Key among these were:

- a shift in focus from energy efficiency in *water recycling* operations, to energy efficiency in *wastewater treatment* operations; and

- the addition of a new project research theme in the form of an emerging wastewater treatment process known as 'aerobic granular sludge' (AGS).

These changes have given the project a more diversified scope and have resulted in enhanced industry impact. These two key project themes are presented separately in this report: the first research theme is presented under the heading "*Towards energy-efficient, low carbon wastewater treatment in Australia*" and the second under the heading "*Assessing treatment performance and carbon emissions profile of aerobic granular sludge*".

Table 1. Synopsis of original RP2017 project outputs and scope, alongside final project scope and outputs.

Original project outputs including scope	Ultimate project outputs including scope
A comprehensive literature review of energy intensity of water recycling operations internationally	A comprehensive literature review of wastewater treatment energy intensity and energy benchmarking methodology internationally
A suite of best practice industry benchmarks for key water recycling technologies	A suite of best practice industry benchmarks for key wastewater treatment technologies
A comprehensive reference library database for project partners to use as a one-stop-shop for reference material relating to low energy/carbon water recycling operations	A comprehensive reference library database for project partners to use as a one-stop-shop for reference material relating to low energy/carbon wastewater treatment and water recycling operations

Submission of journal publications based on project activities	Unchanged
An industry guidance manual on energy benchmarking and optimisation for low carbon/energy water recycling	Information on the relative cost and environmental impacts of state-of-the-art membrane bioreactor wastewater treatment processes coupled to ultraviolet disinfection, as compared to conventional activated sludge coupled to chlorine disinfection
Information on the value of instrumentation (advanced process control and energy sub-metering) in realising energy efficiency savings during water recycling (via real case study assessments of how instruments/ forward control loops have been/can be used to save energy)	Report on energy and carbon emissions performance benchmarking of Australian wastewater treatment operations
Industry guidance manual on the economic implications and benefits of true fit-for-purpose recycled water supply	Information on the start-up, operation, functional ecology and long-term performance of aerobic granular sludge for wastewater treatment
-	Information on the microbial pathogen removal performance of aerobic granular sludge wastewater treatment and implications for downstream water recycling processes
An industry seminar/workshop on low carbon/energy water recycling principles and practises for CRC stakeholders	Seminars at industry and academic forums on wastewater treatment energy benchmarking and aerobic granular sludge
Two PhD graduates	Unchanged

3. Towards energy-efficient, low carbon wastewater treatment in Australia

Introduction to WWTP energy efficiency and energy benchmarking

As above, many of today's wastewater treatment systems are energy-intensive and possess considerable potential for operational and structural optimisation to improve their energy efficiency. The application of 'energy benchmarking' methodology presents opportunities for the water industry to reduce costs by enabling energy savings and energy recovery at WWTPs, whilst at the same time identifying operational issues for WWTP personnel to focus on to improve plant performance and future performance efficiency. Energy benchmarking enables different water utilities to compare their operational energy performance with other utilities or the broader industry, identifying the sources of performance differences for targeted implementation of energy efficiency improvement measures (Krampe & Trautvetter 2012; GHD 2014b). Energy use key performance indicators (KPIs) are developed for a range of wastewater treatment operations and then used by industry to 'benchmark' current treatment process performance and inform subsequent process optimisation needs for future energy efficiency savings (Krampe, 2013). Once best practices are identified, the water industry will set the best practice values as targets for ongoing improvement and efficiency gains (de Haas *et al.* 2015).

The basic premise of energy benchmarking is to collect operating energy consumption data from a given WWTP and then compare (benchmark) this performance level against industry performance benchmark values representing average (50th percentile) and best practice (10th percentile) energy efficiency performance. The difference between the actual energy use and benchmark performance value represents the potential saving to be realised through optimisation (see Figure 1). These 50th and 10th percentile benchmark values are specific to certain technology types (type classes) and sizes (size classes) of WWTPs to ensure proper 'like-for-like' comparisons.

Figure 1. Conceptual overview of WWTP energy benchmarking and potential energy efficiency gains.
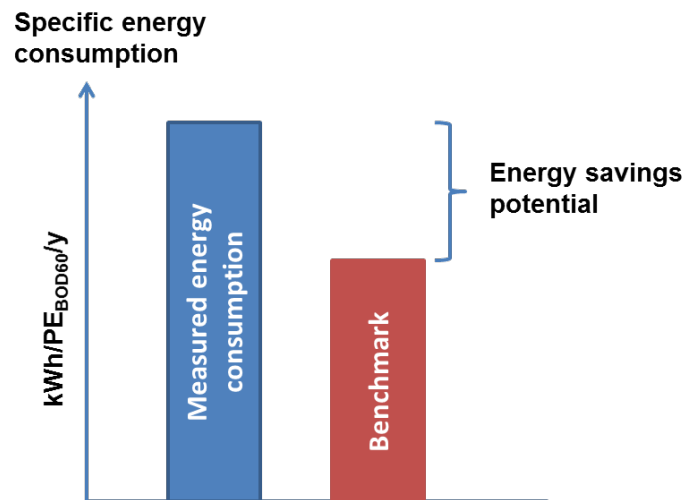
Energy benchmarking today falls under the International Standard ISO 50001:2011 Energy Management Systems (ISO 2011). One of the key activities in both ISO 50001 and energy benchmarking involves the undertaking of an initial energy review to establish an energy performance 'baseline'. This baseline is used for performance monitoring and also set improvement targets in relation to future energy performance. Under ISO 50001:2011 the industry is required to develop, record and maintain an energy review, and document the process. Energy consumption should be analysed based on industry data, with identification of the areas where energy use is significant throughout the facility to determine current energy performance. This can be used to estimate future energy consumption and identify and prioritise opportunities for energy performance improvement, as required. Adjustments to the performance baseline may be made if the performance indicators no longer reflect the industry energy consumption (ISO 2011). The overall framework approach for energy benchmarking is shown in Figure 2. While the ISO 50001 standard provides the overall framework for energy auditing and identifying areas for optimisation, it does not prescribe the energy performance KPIs, nor does it prescribe or recommend a standard/best practice approach to develop them.

Energy benchmarking as applied to wastewater treatment was first developed in Europe in the 1990s and has only recently (circa 2012) begun to be applied by Australian water utilities (Krampe 2013), including two national benchmarking projects coordinated by the water industry's peak body—The Water Services Association of Australia (WSAA)—to date (GHD 2014; GHD 2017). These Australian benchmarking studies have resulted in the collection of a considerable data resource on the energy use profiles of some 244 WWTPs nationally – data that was made available to this project for the development of new and updated WWTP energy benchmarks.

So far, this Australian energy benchmarking work has applied existing European methods and benchmarks to Australian conditions, which in many cases affects the relevance and scale of identified energy efficiency opportunities. For example there are important differences in how wastewater treatment processes perform, the regulated treatment targets and the nature of the wastewater itself which can affect the energy use performance of WWTPs. New locally-relevant energy benchmarks were, therefore, needed for use by the Australian water sector to be able to exploit maximum value from energy efficiency activities and this was a key driver for this component of RP2017 research.

Figure 2. Conceptual overview of WWTP energy benchmarking and efficiency savings.
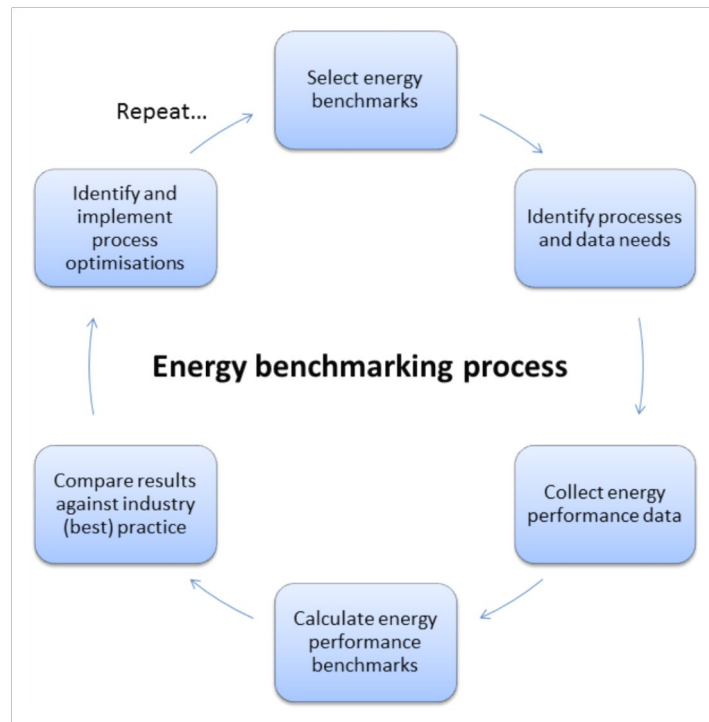
The research objectives and outputs of this project component were:

1. Comprehensive review of energy benchmarking literature and practice internationally;

2. A comprehensive reference library resource for industry on material relating to low energy/carbon wastewater treatment and water recycling operations;

3. A suite of new and updated Australian-relevant energy benchmark KPIs for Australian WWTPs using local industry data;

4. Benchmarking assessment of electricity-related carbon emissions intensity of Australian WWTPs;

5. Provide new information on the relative cost and environmental impacts of state-of-the-art membrane bioreactor wastewater treatment processes coupled to ultraviolet disinfection, as compared to conventional activated sludge coupled to chlorine disinfection for wastewater treatment and water recycling (the "Full-scale WWTP case study investigation").

Brief snapshot summaries of each of these research outputs are given below, with full details for each output presented as separate appendices to the report (where completed and publication restrictions do not preclude inclusion).

Snapshot – Research output 1: Comprehensive review of energy benchmarking literature and practice internationally

The first part of the project has involved a comprehensive, critical review of international energy benchmarking methodology for communication to the water industry both nationally and internationally. Energy benchmarking has been applied internationally and has become common practice in many countries, especially in Europe where the benchmarking methodologies originating in Switzerland and Germany have been widely applied and accepted. There are important differences between these two approaches, however, with the Swiss methodology focused on model WWTP plant-derived theoretical energy

performance value requirements for optimal operating conditions, while the German approach takes a statistical approach based on industry-wide performance data to developing energy performance benchmarks. Beyond German-speaking Europe, the level of understanding surrounding these seminal energy benchmarking approaches has so far been limited. Furthermore, where these methods have been applied internationally, they have in some instances been misinterpreted and improperly applied by the water industry which may hinder the pursuit of best practice WWTP energy efficiency.

This critical review delivers for the first time a complete understanding of the development, evolution and application of seminal European (predominantly German) energy benchmarking methods, unlocking a rich and valuable, but previously inaccessible, knowledge base for an international industry audience. The review also provides detailed summaries of the key information and energy benchmarks required by water industry practitioners to enable them to perform with confidence their own WWTP energy assessment and optimisation activities to help achieve best practice WWTP energy efficiency. As this research output is currently under consideration for publication in the journal *Water Conservation Science and Engineering*, we are unable to provide the complete review text as a report appendix; however, information can be provided on request and interested parties should contact Dr Michael Short (michael.short@unisa.edu.au). The citation for this research output is:

- Clos, I., Krampe, J., Alvarez-Gaitan, J.P., Saint, C.P., Short, M.D. (submitted) Energy benchmarking as a tool for energy efficient wastewater treatment: reviewing international applications with a focus on European methodology. *Water Conservation Science and Engineering*.

Snapshot – Research output 2: Reference library resource on energy efficiency and energy benchmarking in wastewater treatment and water recycling operations

During the course of the project, a substantial amount of literature (scholarly and grey) was reviewed and collated in subject areas relating to energy efficiency and energy benchmarking in wastewater treatment and water recycling operations. A comprehensive reference library with some 420 individual resources has been produced and is provided in Appendix A.

Snapshot – Research output 3: New and updated Australian-relevant energy benchmark KPIs for Australian WWTPs using local industry data

Another phase of the project has involved the use of a comprehensive national WWTP electricity use dataset, collected as part of a national water industry benchmarking assessment coordinated by the Water Services Association of Australia, to develop a suite of locally-relevant, Australian energy performance benchmarks for a range of key wastewater treatment plant sizes and plant types. Performance data collected from each of the 244 WWTP related to the period between July 2015 and June 2016. This suite of new and updated Australian energy benchmarks will enable water industry members to benchmark their energy use performance against their industry's own performance metrics, helping to unlock future energy and GHG emissions savings from wastewater treatment operations. The median (50th percentile) energy performance benchmarks for Australian WWTPs are given in Table 2 for the various WWTP size classes (SC) based on the number of connected population equivalents and plant types (T). Benchmarks are based on WWTP electrical energy use (kWh/year) and integrated with the population equivalent (PE) size calculated from the influent wastewater load to give benchmark units of kWh/(PE×y). Plant types follow the Australian classification of GHD (2017) as follows:

T1 – Activated sludge treatment with separate sludge stabilisation, including those with primary sedimentation, anaerobic digestion (or alternative) and on-site cogeneration (on-site energy produced from biogas). Alternative sludge stabilisation includes: incineration; covered anaerobic lagoons; chemical (e.g. lime) treatment; etc.

T2 – Activated sludge treatment with separate sludge stabilisation, including those with primary sedimentation, anaerobic digestion (or alternative) but without onsite co-generation (no on-site energy produced from biogas). Alternative sludge stabilisation includes: incineration, covered or uncovered anaerobic lagoons; chemical (e.g. lime) treatment; etc.

T3 – Extended aeration activated sludge, including aerobic digestion. Sub-types include:

T3.1 – Compartmentalised (all types, including those for biological nutrient removal configurations) and with clarifiers, but excluding Subtypes 3.2 to 3.5 below;

T3.2 – Oxidation ditch-type activated sludge (including ditches with external compartments such as anaerobic or selector reactors) and with clarifiers;

T3.3 – Intermittent activated sludge processes (e.g. sequencing batch reactors, intermittent decant extended aeration, intermittent decant aerated lagoon);

T3.4 – Membrane bioreactors (MBR);

T3.5 – Moving bed biofilm bioreactors (MBBR), where main aeration zone is MBBR (e.g. excludes tertiary MBBR).

T4 – Trickling filters. Sub-types include:

T4.1 – Trickling filters only;

T4.2 – Trickling filters in combination with activated sludge.

T5 – Lagoon and/or wetland systems. Sub-types include:

T5.1 – Aerated lagoons

T5.2 – Lagoon and/or wetland systems without aeration

As can be seen in the benchmarks in Table 2, energy efficiency generally increases with increasing WWTP size due to recognised economies of scale effects afforded to larger plants relating to increased process and equipment efficiency. Type 4 and 5 WWTPs also tended to have lower energy benchmark values due to the lower technology nature of these systems (trickling filter or lagoon-based). Notable in Table 2 is that many benchmark values are drawn from small sample sizes of <5 WWTPs and so the quality of these benchmark values is low. For comparison, equivalent studies in some European countries have drawn on datasets in excess of 1,000 WWTPs, whereas the current benchmark data are drawn from 244 WWTPs.

The values in Table 2 remain under development and work is ongoing to refine them and develop additional complementary benchmarks for best practice (10th percentile) performance across the full range of WWTP type configurations. Once finalised, these benchmark values can be used by Australian water industry professionals to undertake their own energy benchmarking performance assessments to better understand energy use performance and assess the need for future energy efficiency measures at individual WWTPs.

Table 2. Summary of 50th percentile energy benchmarks (kWh/PE×y) for Australian WWTPs according to plant size class (SC) and plant type (T).

WWTP type	<1000 PE [SC 1]	1000–5000 PE [SC 2]	5001–10000 PE [SC 3]	10001–100000 PE [SC 4]	10001–20000 PE [SC4.1]	20001–50000 PE [SC4.2]	50001–100000 PE [SC4.3]	>1000000 PE [SC 5]
T1	-	-	-	38	37*	43*	38*	38
T2	42*	-	90*	59	57*	56*	56	51
T3.1	231*	135*	125*	59	91*	64	49	44
T3.2	424*	85*	104*	50	63	60	44*	32*
T3.3	132*	86	64*	49	74	51	35	38
T3.4	588*	256	159*	65	279*	72*	54*	-
T4.1	124*	21*	27*	15*	26*	-	-	-
T4.2	-	-	2.4*	47*	25*	54*	39*	-
T5.1	52	70	40	32	47*	42*	-	9.7*
T5.2	53*	13	74*	15*	29*	-	-	-

PE – population equivalent; SC – size class; T – plant type; * – low sample size (<5 WWTPs) so benchmark data quality is considered highly uncertain

Snapshot – Research output 4: Benchmarking assessment of electricity-related carbon emissions intensity of Australian WWTPs

This research output provides a synopsis of Australian WWTP energy benchmarking national assessments undertaken by the water sector, focusing on the two major national benchmarking surveys in 2014 and 2017 date (GHD 2014; GHD 2017). Energy use and, for the first time, electricity-related carbon emissions intensity performance data are presented for wastewater treatment operations covering the vast majority of the Australian population at both a state and national level. National median per capita equivalent specific energy consumption for wastewater treatment was approx. 56 kWh/population equivalent/year, with an associated average per capita equivalent carbon emission intensity of 51 kg CO_2-e/population equivalent/year. The work concludes with a future outlook for best practice WWTP energy performance and benchmarking in the water sector. Full details of this research output are provided in Appendix B. The citation for this research output is:

- Clos I., Alvarez-Gaitan J.P., Saint C.P., Short M.D. (2019) Energy Benchmarking for Efficient, Lower Carbon Wastewater Treatment Operations in Australia. In: Newton P., Prasad D., Sproul A., White S. (eds) *Decarbonising the Built Environment*. Palgrave Macmillan, Singapore. https://doi.org/10.1007/978-981-13-7940-6_16

Snapshot – Research output 5: Full-scale WWTP case study investigation

The best and most sustainable outcome is known to not always be at the technological limit or 'limit of best practice' for water treatment systems, and often the protection of local environmental quality as driven by tighter water sector regulation, comes at a cost of broader environmental impacts (Foley *et al.* 2010). As above, with the shift towards more advanced and highly engineered wastewater treatment processes, comes a need to better understand the full environmental consequences of these technological advancements on the overall treatment system (as compared to the previous conventional treatment norm). Life cycle assessment (LCA) offers a standardised means by which to quantitatively assess the full life cycle environmental performance of products or systems (ISO, 2006).

Working with water industry project partners, this project research component sought to investigate the financial and environmental performance of two wastewater treatment systems using data gathered from full-scale Australian WWTPs as a case study for comparison of state-of-the-art membrane bioreactor (MBR) wastewater treatment technology coupled to ultraviolet (UV) disinfection, as compared to conventional activated sludge (CAS) coupled to chlorine disinfection for wastewater treatment. Working closely with industry stakeholders, comprehensive data were gathered on both the CAS and MBR WWTPs relating to their construction and operation. Data included financial cost and material inventories to enable full economic and environmental assessments to be undertaken. The two WWTPs receive a common influent and are of comparable size and loading (Table 3), allowing for representative comparisons.

Table 3. Summary of wastewater treatment plant size and loading rates for comparison of chlorine versus UV disinfection treatment.

WWTP and disinfection process	Connected population equivalents	Daily flow (ML)	Daily organic load (kg BOD_5)
CAS + chlorine disinfection	85,000	17	3,750
MBR + UV disinfection	65,000	13	2,950

Key summary results of the comparative cost assessment for operation of chlorine versus UV disinfection systems at the case study WWTP are given in Table 4 and Figure 3 below. As shown, flow-normalised costs involved with operating the UV disinfection process at the case study WWTP were substantially greater (some 10-fold higher) than the equivalent chlorine-based disinfection process, owing to the technological

complexity of the UV system. Regarding the percentage distribution of costs, labour (52%) and materials (33%) dominated the cost profile of chlorine disinfection, whereas for the UV disinfection system, labour costs were a comparatively modest fraction of the total (15%), while materials (46%) and energy (36%) dominated the cost profile. Overall, this information will be of value to water industry professionals in the planning of wastewater treatment and water recycling systems, to better understand the costs of these disinfection processes and make informed decisions about the cost-effective delivery of fit for purpose wastewater treatment and recycling operations. While important, cost considerations also need to be considered alongside possible local environmental constraints linked to the use and discharge of residual chlorine to receiving environments.

Table 4. Summary of operating cost data ($AU normalised per megalitre of daily treated wastewater flow) for the full-scale WWTP case study comparison of chlorine disinfection versus UV disinfection, showing breakdown per financial year (during the period 2013–2017) and cost type.

	Financial year	UV disinfection	Chlorine disinfection
Labour	2013	24	525
	2014	765	289
	2015	1,321	298
	2016	3,327	515
	2017	2,197	818
	TOTAL	**7,634**	**2,445**
	Avg. annual	**1,527**	**489**
Materials	2013	212	432
	2014	15	350
	2015	2,677	53
	2016	17,988	150
	2017	2,668	537
	TOTAL	**23,560**	**1,523**
	Avg. annual	**4,712**	**305**
Motor vehicles	2013	-	-
	2014	15	11
	2015	60	9
	2016	190	57
	2017	232	103
	TOTAL	**497**	**181**
	Avg. annual	**99**	**36**
Sub-contractors	2013	-	-
	2014	57	-
	2015	231	-
	2016	27	-
	2017	40	527
	TOTAL	**355**	**527**

	Avg. annual	71	105
Energy	2013	5,195	-
	2014	4,561	-
	2015	2,884	-
	2016	2,868	-
	2017	2,916	-
	TOTAL	**18,425**	**-**
	Avg. annual	**3,685**	**-**
Other	2013	4	7
	2014	47	4
	2015	127	-
	2016	407	1
	2017	116	7
	TOTAL	**701**	**18**
	Avg. annual	**140**	**4**
TOTAL	2013	5,435	964
	2014	5,459	654
	2015	7,300	361
	2016	24,808	723
	2017	8,170	1,993
	TOTAL	**51,172**	**4,695**
	Avg. annual	**10,234**	**939**

Figure 3. Summary of relative cost data for the full-scale WWTP case study comparison of chlorine disinfection versus UV disinfection, showing cost breakdown per cost type and percent contribution to total cost.

Chlorine disinfection (cost breakdown)

- Labour — 52%
- Materials — 33%
- Motor vehicles — 4%
- Sub-contractors — 11%
- Energy — 0.0%
- Other — 0.4%
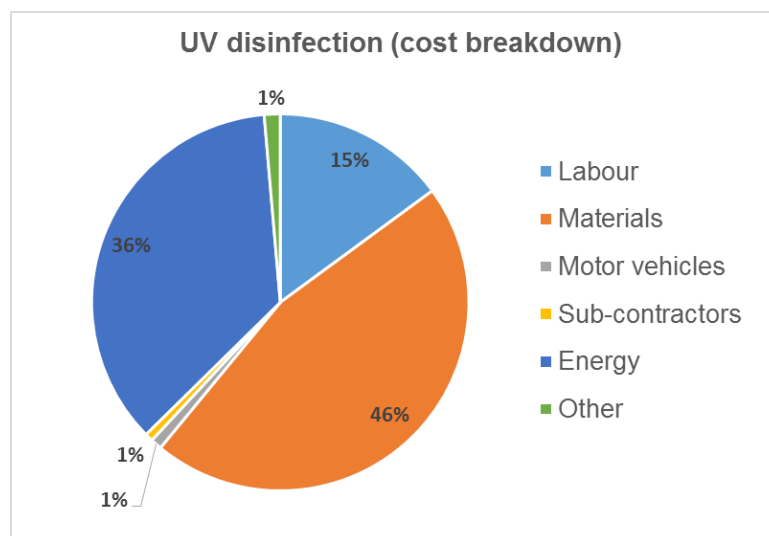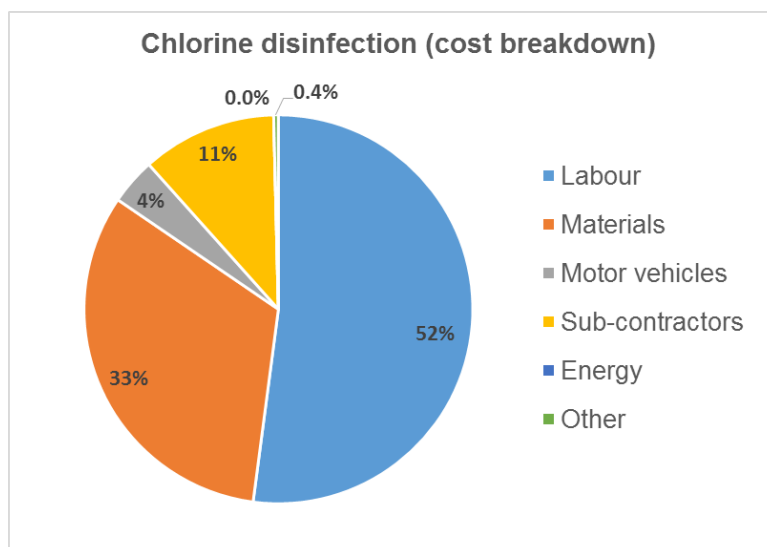
Preliminary results of the comparative environmental life cycle performance assessment for the comparison of conventional activated sludge operation with the membrane bioreactor treatment process are given in Table 5 and Figure 4. Overall, environmental impacts across the seven impact categories suggest lower environmental impacts from CAS operations compared to the MBR process, with the exception of the ozone depletion potential impact category. Lower electricity use during CAS treatment contributed to the approx. 30% lower global warming potential (carbon footprint) in this assessment, but it should be noted that differences in grid electricity supply, and associated electricity emission factor, in other jurisdictions will affect the magnitude of these results. Together with the information on comparative disinfection process costs, results from this research output (once finalised) will provide the water industry with new insights into the economic and environmental performance of key wastewater treatment processes and systems for the sustainable planning and delivery of its future WWTP operations.

The above results for this project output are preliminary since research remains underway as part of an active PhD. Information can be provided on request once research has been finalised and interested parties should contact Dr Michael Short (michael.short@unisa.edu.au).

Table 5. Summary of preliminary life cycle impact assessment results for the full-scale WWTP case study comparison of conventional activated sludge (CAS) with membrane bioreactor (MBR) treatment process (absolute impacts given per unit of 1 m^3 of treated wastewater).

Impact category	CAS	MBR	Unit
Global warming potential - GWP100	91.880	121.41	kg CO_2-equivalents
Fossil depletion potential - FDP	39.777	67.079	kg oil-equivalents
Feshwater eutrophication potential - FEP	0.0319	0.0440	kg P-equivalents
Human toxicity potential - HTP	26.158	31.217	kg 1,4-DCB-equivalents
Marine eutrophication potential - MEP	0.0825	0.1042	kg N-equivalents
Metal depletion potential - MDP	9.7464	10.991	kg Fe-equivalents
Ozone depletion potential - ODP	0.000007	0.000006	kg CFC-11-equivalents

Figure 4. Summary of preliminary life cycle impact assessment results for the full-scale WWTP case study comparison of conventional activated sludge (CAS) with membrane bioreactor (MBR) treatment process (CAS-normalised impacts given per unit of 1 m3 of treated wastewater).
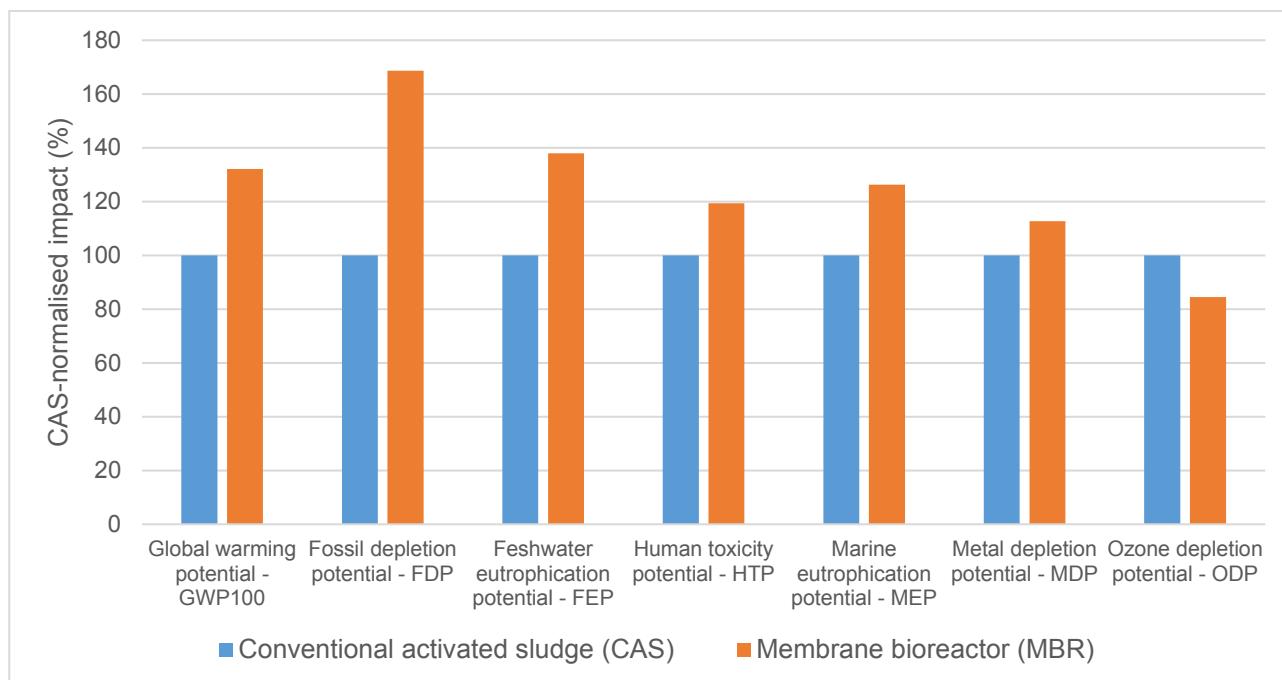
4. Assessing treatment performance and carbon emissions profile of aerobic granular sludge

Background to aerobic granular sludge technology

Aerobic granular sludge (AGS) is a relatively new type of wastewater treatment technology which selects for and uses large microbial granules, as opposed to the current treatment process norm of conventional activated sludge (CAS) which uses small microbial flocs (Figure 5). The benefits of AGS over CAS include:

- Excellent biomass settling (i.e. better separation of solids from water phase);

- Ability to retain and operate higher biomass concentrations (i.e. higher mixed liquor biomass concentrations for enhanced treatment efficacy);

- Potential for shorter reactor operating cycle times which translates to increased WWTP hydraulic capacity;

- Reduced physical footprint of WWTP, with associated possibility of cost savings (capital and operating) and lower energy use requirements.

AGS technology was first discovered in the 1990s, with the first full-scale AGS wastewater treatment facility commencing operation in 2010 in the Netherlands (Wang *et al.* 2017). To date, there has only been one full-scale AGS facility commissioned in Australia, with this facility built in the town of Kingaroy, Queensland (http://www.aquatecmaxcon.com.au/news/268-first-australasian-neredar-plant). The technology is one of emerging interest to the Australian water industry, particularly for retrofitting of existing CAS operations for conversion to AGS. However, despite the advances in our understanding of AGS formation and performance over the past two decades (Bengtsson *et al.* 2018), there are still gaps in our understanding of AGS performance in key areas of relevance for the water industry and several of these gaps formed the basis for this component of the project's research.

Figure 5. Comparison of conventional activated sludge flocs (left) versus aerobic granular sludge granules (right).
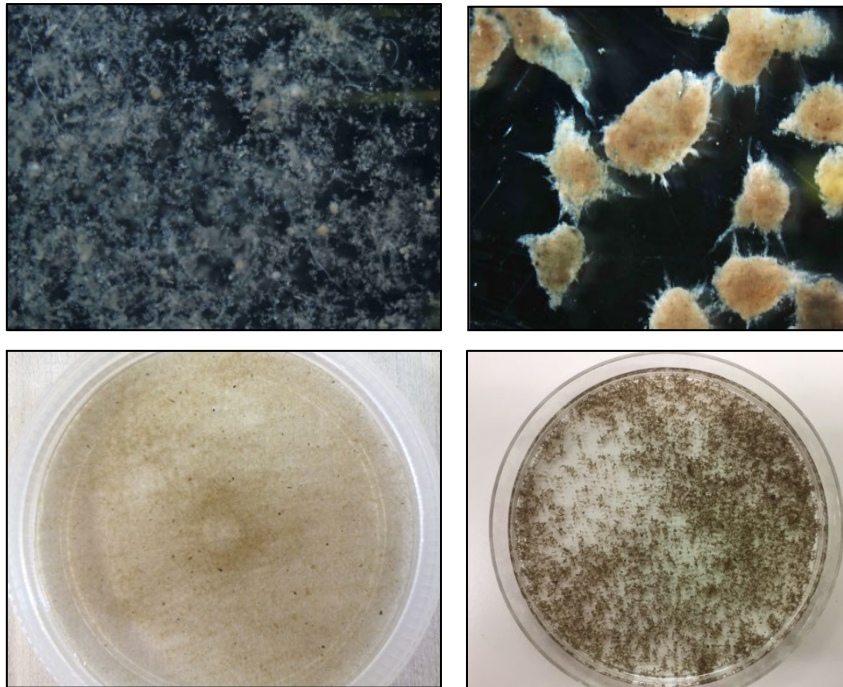
The research objectives and outputs of this project component were:

6. Understanding the role of wastewater feeding strategy (anaerobic or split anaerobic–aerobic) on AGS development and functional performance;

7. Implications of AGS versus CAS operation on microbial pathogen removal performance and the subsequent downstream implications for water recycling operations;

8. Implications for direct process greenhouse gas emissions (nitrous oxide) in AGS versus CAS.

Brief snapshot summaries of each of these research outputs are given below, with full details for each output presented as separate appendices to the report (where possible).

Description of the AGS research facility

A pilot-scale wastewater treatment research facility was constructed and operated by the project's industry partner SA Water at their Bolivar WWTP in Adelaide, South Australia (

Figure 6). This facility consisted of two pilot-scale Perspex sequencing batch reactors (SBRs) with process control via a Siemens programmable logic controller and touchscreen to control cycle times, aeration and other key operating conditions as needed for AGS development. Having two parallel pilot-scale reactors allowed for true side-by-side comparisons of AGS with representative conventional activated sludge operations and at sufficient scale to give industry confidence in the results.

Figure 6. Picture of the pilot research facility at the SA Water Bolivar WWTP site (A) and schematic of the pilot facility operation and process control (B).
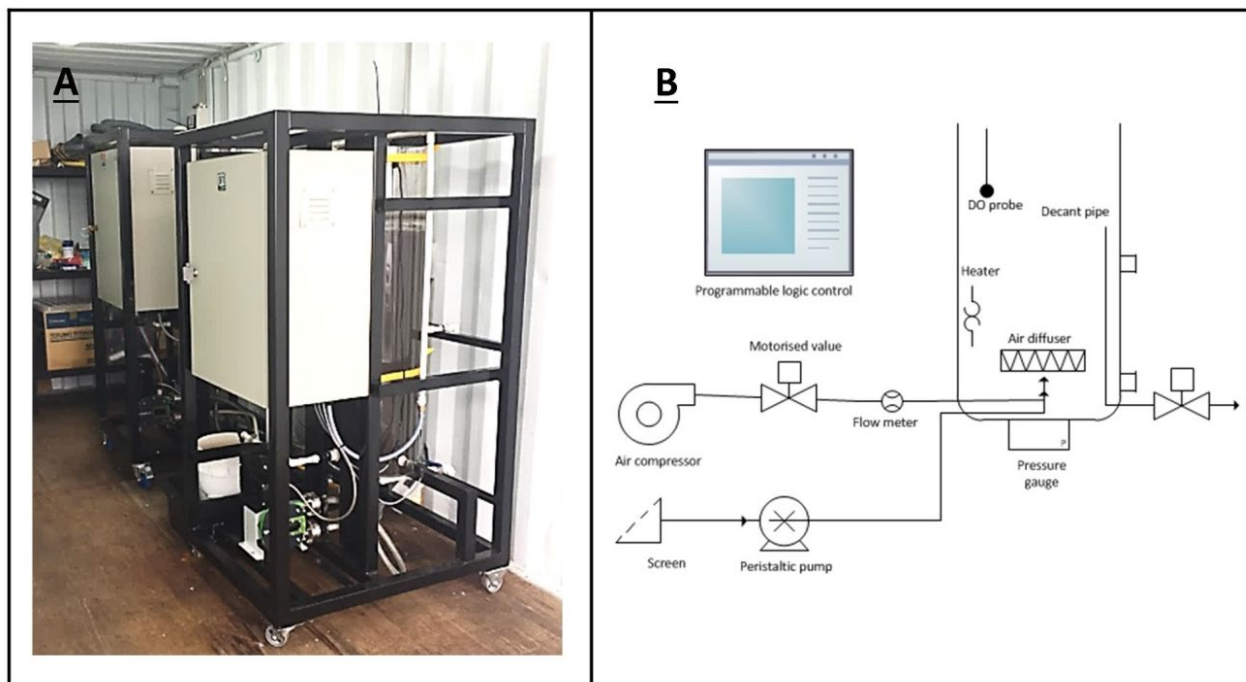
Snapshot – Research output 6: Understanding the role of wastewater feeding strategy (anaerobic or split anaerobic–aerobic) on AGS development and functional performance

The successful development of AGS for wastewater treatment has been linked to a dedicated anaerobic feeding phase, which enables key microbes such as poly-phosphate accumulating organisms and glycogen accumulating organisms to gain a competitive advantage over floc-forming organisms as exist in conventional activated sludge processed. This reliance on a dedicated anaerobic feeding step presents practical and engineering challenges for how SBR wastewater treatment plants are operated, particularly in the context of future retrofitting of AGS to replace conventional activated sludge processes. Research undertaken as part of this project output compared the performance of two feeding strategies (i.e. a split anaerobic–aerobic feed and a traditional dedicated anaerobic feed) and assessed the subsequent performance with regard to AGS formation and stability, nitrogen removal performance and microbial ecology. Results showed that AGS could be established and maintained when using a split anaerobic–aerobic feed at low organic loading rates. Additionally, is was revealed that AGS start-up time and nitrogen removal performance were comparable under both a split anaerobic–aerobic feed and dedicated anaerobic feed. Analyses of the microbial community ecology based on whole-of-community genetic profiling and targeted analysis of functional genes specific for key nitrifying and denitrifying microorganisms, showed that the two different feed strategies had only subtle impacts on both the overall community composition and functional microbial ecology in terms of key nitrifying and denitrifying bacteria and Archaea; however, there were notable ecological differences when comparing different sized AGS granules. In contrast to previous work, a large enrichment in poly-phosphate accumulating organisms in AGS was not observed in the high-saline wastewater, which supported the observation of low phosphate removal performance. AGS biomass was, however, substantially enriched in sulfide-oxidising bacteria, which was complemented by elemental analysis showing the presence of elemental sulfur precipitation within the AGS granules.

Overall, outcomes of this research component demonstrate for the first time that a dedicated anaerobic feed is not universally required for successful AGS development and operation. New insights into the functional microbiology of AGS were also delivered in the context of high saline wastewater treatment, which may have increasing relevance within the context of climate change adaptation for coastal communities in the coming decades. These findings will be of value to water industry members planning to retrofit existing conventional

activated sludge-based SBRs to operate with AGS. Full details of this research output are provided in Appendix C and Appendix D. The citation for these research outputs are:

- Thwaites, B.J., Reeve, P., Dinesh, N., Short, M.D., van den Akker, B. (2017) Comparison of an anaerobic feed and split anaerobic–aerobic feed on granular sludge development, performance and ecology. *Chemosphere* 172: 408–417; https://doi.org/10.1016/j.chemosphere.2016.12.133

- Thwaites, B.J., van den Akker, B., Reeve, P., Short, M.D., Dinesh, N., Alvarez-Gaitan, J.P., Stuetz, R. (2018) Ecology and performance of aerobic granular sludge treating high-saline municipal wastewater. *Water Science & Technology*, 77(4): 1107–1114; https://doi.org/10.2166/wst.2017.626.

Snapshot – Research output 7: Implications of AGS versus CAS operation on microbial pathogen removal performance and the subsequent downstream implications for water recycling operations

Aerobic granular sludge is an emerging treatment technology for both new WWTPs and also as a prospective retrofit technology solution to existing WWTPs for improved treatment performance, enhanced process stability and increased hydraulic capacity. Given its emerging technology status, there are no prior investigations on the ability of AGS to treat and remove microbial pathogens. Such information on microbial pathogen removal performance during wastewater treatment is crucial for the water industry to know in order to be able to maintain adequate downstream treatment and disinfection for public health protection upon effluent discharge to receiving waterways, or during effluent reuse in water recycling schemes. Research done as part of this project output compared the removal performance of commonly used microbial pathogen surrogates (sulfite-reducing clostridia spores, f-RNA bacteriophage, *Escherichia coli* and total coliforms) by AGS and CAS during wastewater treatment operations, from the initial start-up phase, through to mature operation. Results showed that AGS performed as well as CAS for the removal of all microbial surrogates, except for sulfite-reducing clostridia spores which were removed more effectively by AGS than for CAS. This world-first assessment of microbial pathogen removal performance by AGS showed that AGS is capable of meeting or exceeding existing equivalent CAS-based health-based targets for pathogen removal in the context of water recycling. Results also confirmed that AGS operation did not adversely impact the secondary effluent water quality in a way that would have implications for downstream tertiary disinfection processes. Overall, findings from this research output confirmed for the first time that the adoption of AGS operation would not adversely impact water quality in such a way that could impact downstream tertiary disinfection processes or compromise public health protection barriers already in place for CAS systems. These findings provide the water industry with additional confidence in the robustness of AGS-based wastewater treatment processes for both environmental and public health protection. Full details of this research output are provided in Appendix E. The citation for this research output is:

- Thwaites, B.J., Short, M.D., Stuetz, R.M., Reeve, P.J., Alvarez-Gaitan, J.-P., Dinesh, N., van den Akker, B. (2018) Comparing the performance of aerobic granular sludge versus conventional activated sludge for microbial log removal and effluent quality: implications for water reuse. *Water Research*, 145: 442–452; https://doi.org/10.1016/j.watres.2018.08.038.

Snapshot – Research output 8: Implications for direct process greenhouse gas emissions (nitrous oxide) in AGS versus CAS

Nitrous oxide (N_2O) is an important trace gas that plays an important role in several aspects of atmospheric chemistry and climate. N_2O is a both a priority ozone-depleting substance and a potent greenhouse gas (Kanter *et al.* 2013) and the water industry has in recent years, through ongoing international research, become increasingly aware of its importance in particular in relation to the sector's carbon emissions profile (Kampschreur *et al.* 2009; Law *et al.* 2012; Ribera-Guardia *et al.* 2019). So far, there is very little information available internationally on the emissions of N_2O from AGS-based wastewater treatment processes and of the limited available studies, many have used synthetic wastewater and laboratory-scale reactors with unrealistic loading regimes, bringing into question the industry-relevance of the findings. Accordingly, research undertaken as part of this research output investigated the N_2O emissions dynamics of AGS when operated under operationally-relevant loading rates and compared to conventional activated sludge at pilot scale using real municipal wastewater. The changes in N_2O emissions were characterised with regards to

operational conditions such as nitrogen loading and dissolved oxygen concentration, and were contrasted with N_2O respective emissions from parallel conventional activated sludge operation.

Results showed that the removals of incoming ammonia nitrogen and organic load (as measured by chemical oxygen demand) were comparable in both AGS and CAS reactors at 99% and 90% respectively. Regarding N_2O emissions, results showed that when the reactors were operated at low organic loading rates of <0.6 kg chemical oxygen demand/m^3/d, the N_2O emissions were comparable between AGS and CAS (Figure 7). However, exceeding this loading of 0.6 kg chemical oxygen demand/m^3/d resulted in an increased N_2O production by AGS relative to CAS. It was unclear from the study to what extent the higher observed N_2O emissions from AGS were a result of the pilot-scale operation and subsequent aeration process control limitations, so it was suggested that future work should look to further understand the impact of this effect, or ideally test emissions from full-scale reactors. Overall, findings from this research output have expanded our knowledge on the N_2O emissions consequences of AGS, knowledge which will help the water industry better understand the full environmental consequences of any future transition from CAS to AGS-based wastewater treatment processes.

The results for this research output are preliminary since research under this project output currently remains underway as part of an active PhD. Information can be provided on request once research has been finalised and interested parties should contact Dr Michael Short (michael.short@unisa.edu.au).

Figure 7. Total N_2O-N flux (*y*-axis; grams N_2O-N/hour/m^2) versus ammonium loading rate (kg NH_3-N/m^3/day) for CAS and AGS reactors. Encircled data are considered erroneously high due to thermal effects from reactor temperatures exceeding 25°C.
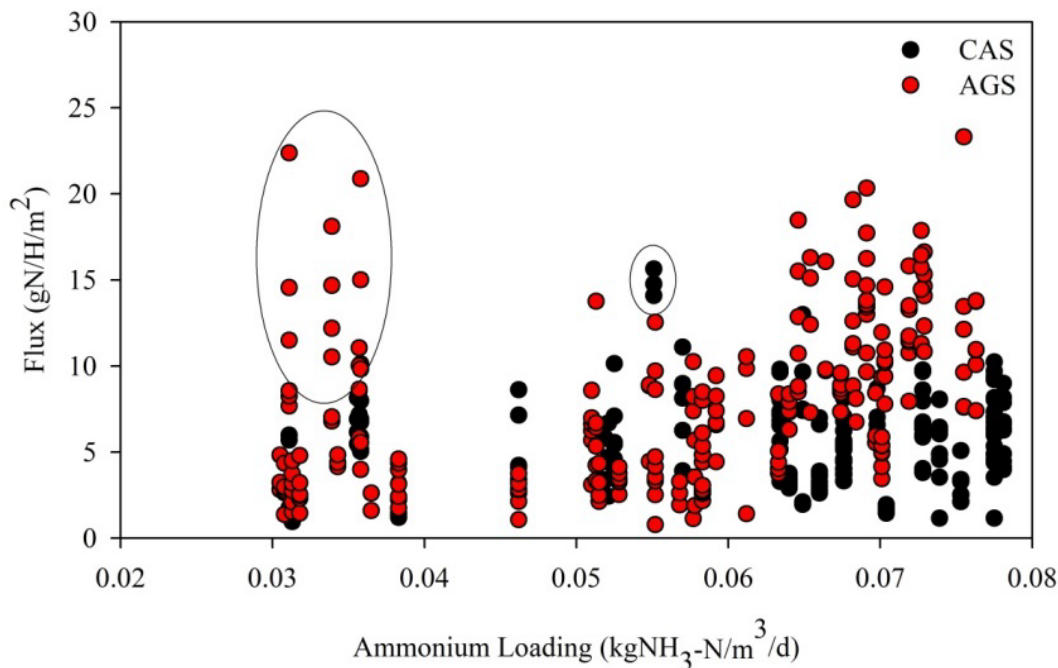
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5. Appendices

Appendix A. Research output 2: Reference library resource on energy efficiency and energy benchmarking in wastewater treatment and water recycling operations

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Title: Energy benchmarking for efficient, lower carbon wastewater treatment operations in Australia

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Abstract: Wastewater treatment operations are energy-intensive and often require operational and design optimisation to improve their energy efficiency. The application of an energy benchmarking approach presents opportunities for wastewater treatment plants (WWTPs) to reduce costs by enabling energy savings and energy recovery, whilst at the same time identifying operational issues for WWTP personnel to focus on to improve plant performance. Energy benchmarking broadly seeks to help the water sector identify and adopt best practice efficiency in the pursuit of better industry performance. Energy benchmarking methodology has emerged from central Europe since the mid-1990s and is now common practice in many countries, especially in Europe where such methods are now widely applied as accepted industry practice. This chapter begins with an introduction to energy efficiency and management in wastewater treatment. This is followed by a synopsis of Australian energy benchmarking and optimisation efforts to date following European methodologies, including two national WWTP energy assessments conducted by the water sector in 2014 and 2016 respectively. The chapter finishes with an assessment of electricity-related greenhouse gas emissions from Australian wastewater treatment operations since energy benchmarking efforts begun and a future outlook for best practice WWTP energy performance and benchmarking in the water sector.

Keywords: energy benchmarking; energy use efficiency; energy management systems; greenhouse gas emissions; ISO 5001; wastewater treatment.

Introduction

Due to the progressive development and implementation of ever more stringent human and environmental health regulations throughout the second half of the 20th century, the water industry has largely been focused on meeting wastewater treatment and effluent quality criteria for proper regulatory compliance (Jenkins & Wanner, 2014), with less emphasis placed on efficiency and innovation in its operations until quite recently. The same is largely true of energy use for wastewater treatment.

Historically, energy has been relatively inexpensive internationally and many wastewater treatment facilities were not designed or operated with energy use efficiency in mind (NYSERDA, 2010). Moreover, the gradual progression from simple low-cost treatment processes, to more advanced highly engineered processes in order to meet increasingly stringent regulatory criteria, has led to a progressive increase in the energy intensity of wastewater treatment over time (Chang et al., 2008). This progressive intensification of energy demands for wastewater treatment has been brought sharply into focus in recent years by dramatic increases in the unit cost of energy, including electricity (AEMO, 2016), as well as increasing volatility in energy tariffs linked to deregulation and structural changes to energy markets (Escribano et al., 2011). Increased environmental awareness and the relevance of energy use and greenhouse gas emissions has also progressively driven the need for energy efficiency and process optimisation in wastewater operations. In combination, these factors have increased the pressure on energy-intensive industries and facilities like wastewater treatment plants (WWTPs) to look for ways to minimise operational energy use.

Energy use and efficiency in wastewater treatment

While energy required for wastewater treatment on a per capita basis is some 10-fold lower than that of domestic water heating for example (Kenway et al., 2015), WWTPs as industrial facilities are typically among the largest single energy users of municipalities (Krampe, 2013; Müller et al., 2010), thereby presenting important opportunities for energy optimisation and efficiency gains. WWTPs can represent one seventh (1/7th) of the total energy consumption of municipal public structures and facilities, with energy also constituting some 20–40% of total WWTP operating costs (US EPA, 2013).

Inefficiencies in WWTPs are due to various factors, including: use of inefficient equipment, usually from the over design of pumps and processes; incorrect operational practices and/or lack of proper controls; and a lack of operator understanding of energy conservation measures (Chang et al., 2008; Ragazzo et al.,

2015). Also, the recent adoption of energy-hungry 'state-of-the-art' technologies such as membrane bioreactors and UV disinfection has become increasingly common, in some cases without proper justification for such advanced technologies (Ragazzo et al., 2015). Practically all WWTPs present opportunities for energy savings, including—or perhaps especially—new plants (Müller et al., 2010). To improve energy efficiency in the water sector, energy benchmarking has been applied internationally with the broad goal of helping the sector identify and adopt best practice efficiency in the pursuit of better industry performance (Cabrera et al., 2011).

ISO 50001: Energy Management Systems

Energy benchmarking in the water sector is a sub-set of the broader benchmarking approach and falls under the International Standard ISO 50001:2011 Energy Management Systems (ISO, 2011). Energy benchmarking enables different water utilities to equate their operational energy performance with other water utilities and comparatively measure their performance, as well as identifying the source of differences for targeted implementation of energy efficiency improvement measures (GHD, 2014b; Krampe & Trautvetter, 2012). Once best practices are identified, the water industry then sets the best practice values (so-called Target Values) for ongoing improvement and efficiency gains (de Haas et al., 2015).

One of the key activities in energy benchmarking involves the undertaking of an initial energy review to establish an energy performance baseline. This baseline is then used for ongoing performance monitoring and setting improvement targets in relation to future energy performance. Adjustments to this baseline may be made if the performance indicators no longer reflect the industry energy consumption (ISO, 2011). Under ISO 50001:2011, the industry is required to develop, record and maintain an energy review, and document the process. Energy consumption should be analysed based on industry data, with identification of the areas where energy use is significant throughout the facility to determine current energy performance.

While ISO 50001 provides the overall framework for energy auditing and identifying areas for optimisation, it does not prescribe the energy performance indicators (benchmarks) nor does it prescribe or recommend a standard/best practice approach to develop them. This leaves the water industry to determine the best approach for energy benchmarking and the setting of energy performance benchmarks. The first European energy benchmarking manual was developed in Switzerland in the mid-1990s and since then, considerable effort has gone into developing and refining these methods, with European methodology now considered world's best practice (Crawford, 2010) and today embraced and replicated in many other countries, including Australia.

Australian energy benchmarking in wastewater treatment

In 2006, the Commonwealth Government of Australia established an Energy Efficiency Opportunities (EEO) program (enacted by the EEO Act 2006) to encourage industry and commercial sectors to pursue cost-effective energy efficiency initiatives. An essential function of the EEO program was the undertaking of a rigorous/comprehensive assessment of energy use, the purpose being to identify cost-effective energy savings with a payback period of up to four years. Participation in the program was compulsory for businesses that individually, or as part of a corporate group, had energy use >0.5 PJ/y. As at June 2013, EEO member corporations accounted for 56% of Australia's total energy use (Australian Government, 2006, 2010); however, the EEO program was closed in 2014 with the repeal of the EEO Act.

Following on from its first EEO report and energy baseline in 2009 (SA Water, 2009), the South Australian water utility SA Water undertook the first ever Australian energy benchmarking assessment of its wastewater treatment operations in 2012, with 24 WWTPs subject to detailed assessments (Krampe & Trautvetter, 2012). The study followed the German methodology (Müller et al., 1999), incorporating benchmark optimisation values from Baumann and Roth (2008) and Haberkern et al. (2008) to enable a wider variety of treatment processes and WWTP sizes to be captured (Krampe & Trautvetter, 2012). The methodology followed the same WWTP size classifications as determined in German benchmarking methodology for consistency with the benchmarks of Baumann and Roth (2008) and Haberkern et al. (2008).

This pioneering benchmarking work from South Australia recognised that the European benchmarks may not be fully applicable to Australian contexts; e.g., due to higher nitrogen loads in Australian wastewater (Krampe, 2013). The energy requirements of nutrient-removing WWTPs is strongly dependent on the nitrogen-to-organic carbon (N:COD) ratio in the raw wastewater, due to the oxygen consumption for nitrification and

also because of the need for reduced COD removal by primary sedimentation in the case of a high N:COD ratio (Nowak, 2003). Nevertheless, the effluent targets between Europe and Australia were considered to be comparable (Krampe, 2013). Despite some issues with data coverage quality, this initial energy benchmarking work was extremely useful and helped to identify significant potential for energy efficiency optimisation, whilst also identifying data gaps for future such assessments (Krampe, 2013).

Following South Australia's lead, in 2012 the Australian water industry peak body (the Water Services Association of Australia; WSAA) conducted an energy survey with the participation of 16 water utilities. This first national energy survey captured 134 WWTPs, recording a total energy consumption of approx. 16 GWh/y (Krampe, 2012). Based on this initial survey, the first national Australian energy benchmarking assessment commenced in 2013. The study involved the collection of data from 17 water utilities spread across seven states and territories, including in total 142 WWTPs (GHD, 2014a, 2014b). The study applied the same approach of SA Water (Krampe and Trautvetter, 2012; Krampe, 2013) and based its evaluation on 2013–2014 financial year data.

Results showed that 10% of assessed WWTPs had energy efficiency performance close to the best practice Target Values (GHD, 2014a) (as specified by Baumann and Roth (2008) and Haberkern et al. (2008)) – a good outcome given that Target Values represent 10th percentile energy performance in category. When referring to Guide Value performance (50th percentile), Australian WWTPs performed significantly below expectations, with only 16% approaching these values (GHD, 2014a, 2014b) and highlighting the substantial future scope for energy efficiency improvements. Usefully, this initial study identified the minimum requirements for data collection, serving as a useful guide to water utilities in future energy optimisation efforts. It also provided a good baseline for understanding and improving future energy benchmarking and performance assessments by providing a reference manual for water utilities on to how identify WWTPs that represent best opportunity for energy efficiency improvements.

In 2017 a second study was commissioned by WSAA, this time evaluating 245 WWTPs across Australia and New Zealand. The results showed that although there had been improvement in data recording and collection and overall WWTPs showed improvement in energy efficiency (when compared to 2014 data), there was still much more to be done to improve energy performance and refine energy benchmarks (de Haas et al., 2018; GHD, 2017).

Overview of national WWTP energy performance assessments

The 2016–17 survey by WSAA gathered information relevant to energy benchmarking analysis, including: general information (name, location and design capacity of WWTP expressed as megalitres (ML)/d and kg BOD_5/d, overall process description, pumping head); secondary effluent quality (chemical oxygen demand (COD), ammonia- and oxidised-nitrogen – all in mg/L); influent loads (flow in ML/d, COD in kg/d, total nitrogen (TN) load in kg/d); biogas production and on site power generation (biogas volume produced in ML/y or m^3/y, amount of biogas wasted/flared in ML/y, electricity generated from biogas in MWh/y, analysis data of the heat value of biogas or the % methane content); energy consumption of the plant (total electricity consumed, total external fuel source consumed, electricity consumption for the aeration of the secondary treatment stage). These data were assessed and a results summary of energy performance for the Australian state and territories, considering WWTP size and operational configuration, is presented below.

Some 87% of the Australian population is connected to sewage systems (UNSTAT, 2011), or approx. 21.8 million people. There are 74 Australian urban water utilities with a combined 673 municipal WWTPs (Bureau of Meteorology, 2018) collecting a combined wastewater volume of 1,896,641 ML during the 2015–16 period (ABS, 2017). The WSAA benchmarking study captured data from 245 WWTPs, 243 of which were Australian and the remainder from New Zealand. This chapter deals only with the performance of Australian WWTPs. These 243 WWTPs have a total annual operational capacity of 24,659,180 PE_{COD}[1], with a total treated wastewater volume of 1,528,210 ML, or some 4,185 ML/d. Though representing some 36% of all WWTPs

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[1] Number of connected population equivalents is expressed as the sum of population pollution load in domestic wastewater (served inhabitants) and the measured pollution (organic) load from commercial sources entering a sewage treatment plant). A standard population-specific COD load of 120 g/PE/d was applied.

nationally, the 243 Australian WWTPs surveyed include the largest metropolitan plants and so collect and treat around 81% of the total national sewage flow (ABS, 2017).

The assessment of WWTPs was carried out according to predefined WWTPs size classes (SC) and the distribution of WWTPs per SC is shown in Figure 1. Notably, plants in SC 5 (>100,000 population equivalents; PE), while representing only 16% of the surveyed WWTPs, are responsible for 81.6% of WSAA surveyed wastewater flow treated (1,209,151 ML) or some 63.8% of the total treated wastewater flow nationally. In addition to size class, the WWTPs were assessed according to the plant's process configuration typology, being plant Types 1 to 5 (GHD, 2014b, 2017). When assessed according to WWTP process configuration or type, 133 of the total 243 WWTPs (≈55% of total) were classified as Type 3 extended aeration activated sludge systems. The next most common process types were Type 5 aerated lagoons with 52 WWTPs (21.3% of total), Type 2 activated sludge systems with separate sludge stabilization but without on-site biogas co-generation with 24 WWTPs (9.8% of total), Type 1 activated sludge systems with separate sludge stabilization and on-site biogas co-generation with 22 WWTPs (9% of total) and Type 4 trickling filters with 13 WWTPs (5.3% of total surveyed plants).
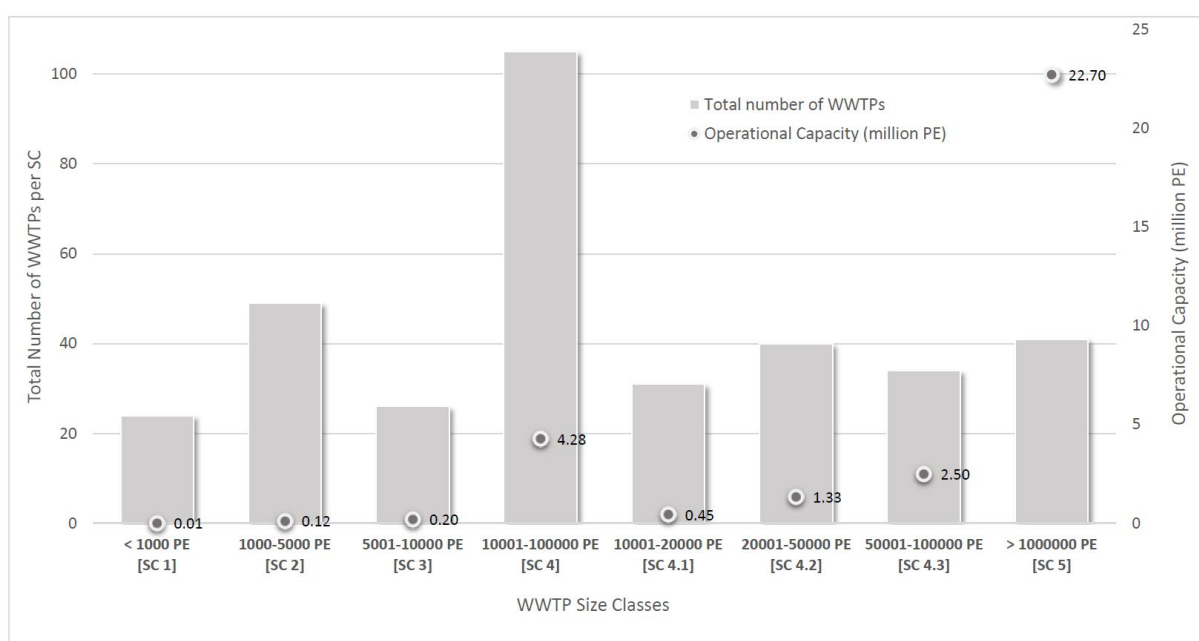

Figure 1: Breakdown of WWTPs surveyed in 2016 benchmarking analysis according to size classification.

Wastewater treatment performance results

Table 1 summarizes the 2016 national WWTP load and performance characteristics per Australian state and territory, including population equivalent-normalised wastewater volumes, pollutant loads, electricity use performance, and related carbon dioxide-equivalent (CO_2-e) emissions. Here, it should be noted that industrial WWTPs (pulp, pharmaceutical and leather industries) are also included, each of which contain a high proportion of hard-to-degrade COD in the wastewater.

Type 3 extended aeration activated sludge systems were shown to have the lowest effluent COD discharge values on average, achieving COD removal ratios of 66.1–99.2% (median 95.5%), followed by Type 1 activated sludge systems with COD removals of 88.8–97.7% (median 93.85%), Type 2 activated sludge systems with COD removals of 90–98.2% (median 93.37%), and Type 5 aerated lagoons with the poorest COD removals at 46.9–93% (median 87.2%).

In the case of total nitrogen, the Type 3 activated sludge systems achieved the best results with a median effluent value of 5.3 mg/l (range 1–30.16 mg/l) followed by Type 2 activated sludge systems with a median effluent value of 8.15 mg/l (range 2.39–40.69 mg/l). Type 5 aerated lagoon WWTPs [T5] achieved median effluent TN levels of 11.72 mg/l (range 2.10–76.25 mg/l), followed by Type 1 activated sludge systems

with median effluent TN of 16.02 mg/l (range 3.70–57.62 mg/l) and lastly Type 4 trickling filters which achieved a median effluent TN of 30.04 mg/l (range 3.77–44.12 mg/l).

Regarding energy use efficiency, trickling filters displayed the best energy performance with a median of 30.7 kWh/(PE/y) and associated carbon emissions 27.9 kg CO_2-e/(PE/y), with Type 3 extended aeration activated sludge systems having the highest median specific electricity use of 62.5 kWh/(PE/y) and associated carbon emissions intensity of 56.8 kg CO_2-e/(PE/y).

Table 1: Summary of 2016 national performance data for all 243 Australian WWTPs surveyed.

	ACT[1]	QLD	NSW	SA	TAS	VIC	WA	Australia
WWTPs surveyed	2	61	48	12	10	89	21	243
Wastewater flow (million m³/y)	33.12	261.9	583.0	54.88	23.89	427.2	144.2	1,528
Operational capacity (million PE)	0.49	4.36	7.75	0.93	0.45	8.37	2.31	24.66
Specific wastewater flow [m³/(PE/y)]	66.9	67.29	74.3	76.61	55.29	73.08	86.77	73.08[2]
Influent COD (mg/l)	655.2	726.3	572.6	752.6	771.3	648.4	800.4	726.3[2]
Effluent COD (mg/l)	12.16	35.55	156.7	67.93	-	40.99	29.11	38.27[2]
COD removed (%)	98.14	94.73	91.8	89.42	-	95.06	95.23	94.89[2]
Influent TN (mg/l)	81.97	62.27	58.68	87.76	58.63	64.23	68.43	64.23[2]
Effluent TN (mg/l)	14.95	3.78	7.38	8.82	28.84	7.05	14.10	8.82[2]
TN removed (%)	78.32	93.39	86.58	84.93	51.95	88.40	74.45	84.93[2]
Specific energy consumption [kWh/(PE/y)]	317.1[3]	59.06	56.10	48.80	41.80	51.91	57.36	56.10[2]
Flow-specific energy consumption (kWh/m³)	4.76[3]	0.84	0.78	0.75	0.68	0.78	0.83	0.78[2]
Nutrient-specific energy consumption (kWh/kg TN removed)	137.8[3]	16.51	16.80	11.64	27.19	15.94	18.08	16.80[2]
Carbon dioxide equivalent emissions [kg CO_2-e/(PE/y)][4]	298.5[3]	54.34	52.73	27.77	7.52	60.22	43.59	51.05[2]

[1] Australian States and Territories: ACT – Australian Capital Territory; QLD – Queensland; NSW – New South Wales; SA – South Australia; TAS – Tasmania; VIC – Victoria; WA – Western Australia; [2] Average data; [3] Data considered non-representative of true performance due to very small sample size (n = 2); [4] State-based grid electricity emission factors (kg CO_2-e/kWh; full fuel cycle scope 2 + 3) sourced from Australian Government (2018).

WWTP energy efficiency and carbon emissions trends

Referring to 2016 national WWTP performance data (Table 1), Australian national average specific energy performance was 56.1 kWh/(PE/y) and per capita equivalent greenhouse gas emissions some 51.1 kg CO_2-e/(PE/y). Total WWTP annual energy use in both 2014 and 2016 survey years and total associated carbon emissions is shown in Figure 2. The energy use patterns for wastewater treatment operations largely reflect state population sizes, with the performance of NSW disproportionately lower than its relative population size due to several large capacity primary-only treatment WWTPs (combined PE of these primary-only plants is some 4 million). Overall, total WWTP energy use and carbon emissions were relatively consistent between 2014 and 2016 survey years, with the exception of South Australia which achieved an approx. 50% reduction in both total annual energy use and carbon emissions due to significant investment in WWTP process efficiencies and optimisation.

Figure 3 gives the per capita equivalent specific energy use and associated carbon emissions intensity of WWTPs in both 2014 and 2016 survey years according to state. At the national average level, specific energy use efficiency of these plants improved overall by some 13% from 54.7 kWh/(PE/y) in 2014 to 47.5 kWh/(PE/y) in 2016. At the state level, most states performed similarly to the national average values. Notable exceptions were Tasmania which performed best in terms of both specific energy consumption (41.8 kWh/(PE/y)) and per capita equivalent greenhouse gas emissions (7.52 kg CO_2-e/(PE/y)), with the very low carbon emissions intensity there due to the predominance of hydroelectricity in this state. South Australia was the next best performer for both specific energy consumption (44.8 kWh/(PE/y)) and per capita equivalent greenhouse gas emissions (27.8 kg CO_2-e/(PE/y)). Large differences in carbon emissions intensity performance between states are a reflection of differing WWTP specific energy performance combined with variable state-based emission factors for grid electricity.
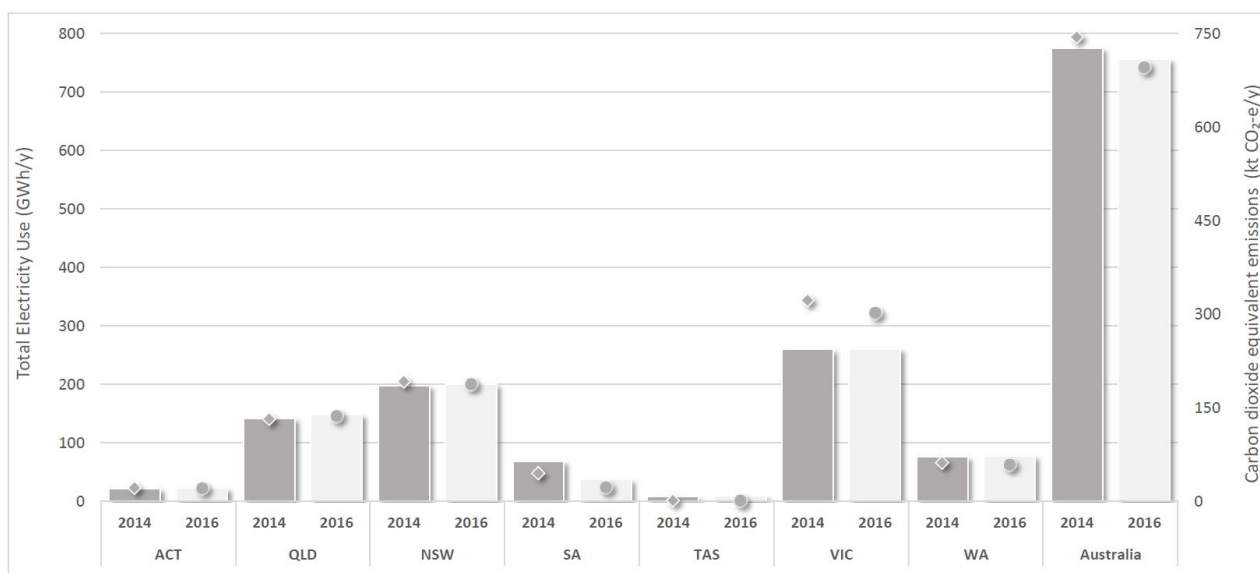

Figure 2: Australian WWTP total annual electricity use (GWh; histogram bars) and carbon dioxide equivalent emissions (kt CO_2-e; ♦, ●) per state for survey years 2014 and 2016 respectively. Data derived only from those 121 WWTPs participating in both survey years.
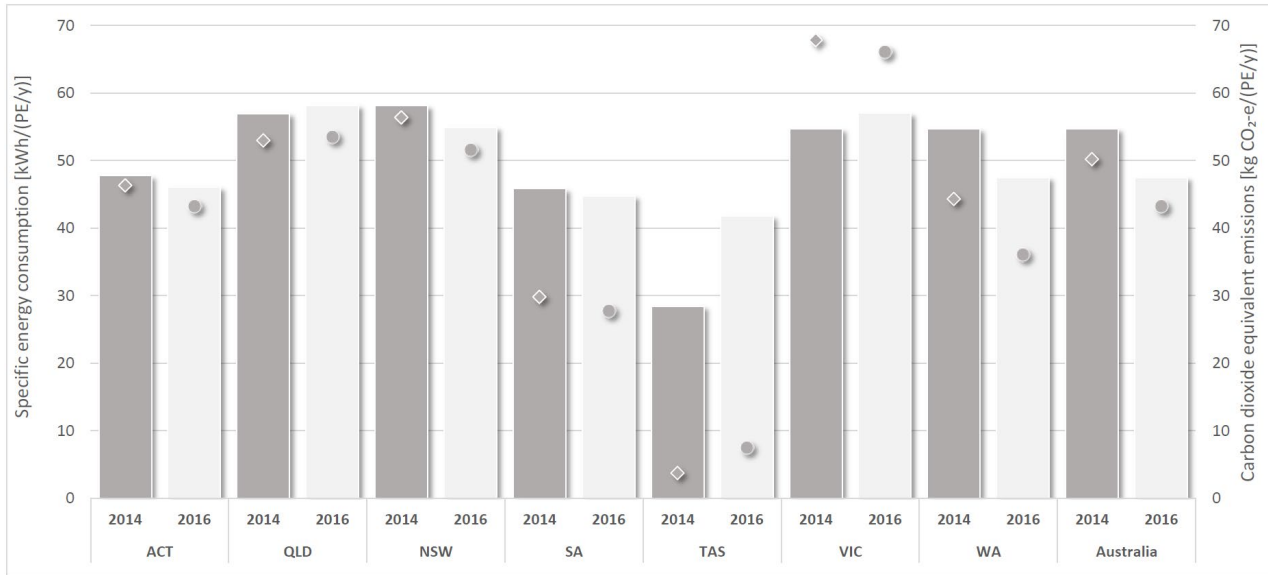
Figure 3: Australian WWTP specific electricity use (kWh/PE/y; histogram bars) and carbon dioxide equivalent emissions (CO_2-e/PE/y; ♦, ●) per state for survey years 2014 and 2016 respectively. Data derived only from those 121 WWTPs participating in both survey years.

Future outlook for energy efficiency and low carbon wastewater treatment

The Australian water industry has invested considerable resources toward energy efficiency initiatives in recent years and many water authorities now recognise the important role of optimising wastewater treatment operations in achieving their corporate energy and carbon neutrality objectives. This chapter has presented a summary of WWTP energy benchmarking work to date, with WWTP energy use and carbon emissions intensity performance data given for wastewater treatment operations covering the majority of the Australian population. National median per capita equivalent specific energy consumption was some 56 kWh/(PE/y), with an associated average per capita equivalent carbon emission intensity of 51 kg CO_2-e/(PE/y). While wastewater treatment operations are a dominant source of greenhouse gas emissions for the water industry, greenhouse gas emissions from WWTPs are a relatively minor component of the total national CO_2-e emissions inventory, contributing <1% to the total inventory.

The undertaking of energy benchmarking and subsequent WWTP energy efficiency optimisations have delivered measurable gains for some state water authorities in recent years; however, considerable scope exists to further optimise WWTP processes for future energy and carbon reductions. Participation in national energy benchmarking projects is currently voluntary, but international experience has demonstrated the importance of comprehensive industry participation in benchmarking exercises to develop robust performance metrics and ensure industry gets the most from benchmarking efforts. Regular and consistent updates of energy benchmarks are also required to ensure that they reflect current industry best practice, technological advancements and regulated environmental performance criteria.

Acknowledgements

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Appendix C. Research output 6: Understanding the role of wastewater feeding strategy (anaerobic or split anaerobic–aerobic) on AGS development and functional performance

This research output has been published in the following:

Title: Comparison of an anaerobic feed and split anaerobic–aerobic feed on granular sludge development, performance and ecology

Authors: Benjamin J. Thwaites, Petra Reeve, Nirmala Dinesh, Michael D. Short, Ben van den Akker

Abstract: The retrofitting of existing wastewater sequencing batch reactors (SBRs) to select for rapid-settling aerobic granular sludge (AGS) over floc-based conventional activated sludge (CAS), could be a viable option to decrease reactor cycle time and increase hydraulic capacity. Successful CAS-to-AGS conversion has previously been shown to be highly dependent on having a dedicated anaerobic feed, which presents additional engineering challenges when retrofitting SBRs. In this study we compared the performance of a split anaerobic–aerobic (An–Aer) feed with that of a traditional dedicated anaerobic feed regarding AGS formation and stability, nitrogen removal performance and microbial ecology. Using pilot trials, we showed that AGS could be established and maintained when using a split An–Aer feed at low organic loading rates analogous to that of a parallel full-scale conventional SBR. Additionally, we showed that AGS start-up time and nitrogen removal performance were comparable under a split An–Aer feed and dedicated anaerobic feed. Microbial ecology characterisations based on whole-of-community 16S rRNA profiles and targeted analysis of functional genes specific for nitrifying and denitrifying microorganisms, showed that the two different feed strategies had only subtle impacts on both the overall community composition and functional ecology. A much greater divergence in microbial ecology was seen when comparing AGS with CAS. Data presented here will be of value to those planning to retrofit existing CAS-based SBRs to operate with AGS and demonstrates the viability of using a more cost-effective split An–Aer feed configuration over a dedicated anaerobic feed.

Highlights

- Two feeding strategies were compared for aerobic granular sludge SBR operation
- Performance of split anaerobic–aerobic and dedicated anaerobic feeding was compared
- Start-up, performance and microbial ecology were similar under both feed strategies
- Aerobic granular sludge ecology was different to conventional activated sludge
- Split anaerobic–aerobic feeding is a viable, cost-effective SBR retrofit solution

1. Introduction

Aerobic granular sludge (AGS) is an emerging technology for secondary-level wastewater treatment which utilises rapid-settling microbial granules in place of floc-based conventional activated sludge (CAS). Selection for the development of AGS is commonly achieved using sequencing batch reactors (SBRs). Two key conditions are typically required for AGS establishment: an anaerobic feed; and a short settling time (<30 minutes) which ensures effective washout of fast-growing, floc-forming organisms (Morgenroth et al., 1997). The anaerobic feed selects for microorganisms such as polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) which store available organic carbon and, therefore, gain a competitive advantage over floc-forming organisms and filamentous bacteria (Bassin et al., 2012, Beun et al., 1999, Liu and Liu, 2006). One of the main advantages of AGS technology is the rapid settling time which results in shortened settling times in SBRs. The cycle saving can be utilised to increase the hydraulic capacity of an existing WWTP or reduce the geographical footprint of new SBRs.

The decreased settling time and denser microbial structure increases the concentration of biomass within the reactor, this provides greater nutrient removal capacity and potential to tolerate increased organic loading and resilience to shock loading events (Long et al., 2015).

The conversion of slow-settling flocs into rapid-settling granules was previously shown to be highly dependent on a long anaerobic feed, as opposed to an aerobic feed which is commonly employed in the operation of most existing secondary treatment SBRs (Beun et al., 1999, de Kreuk et al., 2005a). For newly built granular sludge SBRs, the anaerobic feed can be coupled with the decant phase (Pronk et al., 2015b, de Kreuk, 2006). Here the wastewater is fed in plug flow from the bottom of the reactor through the settled biomass bed, allowing extended anaerobic contact while the treated effluent above is simultaneously decanted from the reactor surface (Pronk et al., 2015b). Shorter cycle times are consequently achieved when coupling the anaerobic feed and decant phases. When retrofitting existing SBRs to operate with AGS, the use of a simultaneous anaerobic feed and decant presents engineering challenges and can be expensive to retrofit, as it requires changes to the inlet design and decant weir to ensure plug flow conditions. For existing SBRs that cannot be reconfigured to operate under simultaneous feed and decant conditions, the use of a separate dedicated anaerobic feed at the beginning of the cycle is in principle the most feasible approach to achieve AGS. However, this approach provides no net reduction in total cycle time, as the savings gained by the shorter settling phase are effectively offset by the addition of a dedicated anaerobic feed step. Alternatively, the use of a split anaerobic–aerobic (An–Aer) feed is more easily achieved within existing SBR configurations and would still yield net cycle time savings; however, the impact of this feed strategy on AGS formation and stability is unclear.

The proposed use of a split An–Aer feed to achieve AGS may be especially suited to wastewater treatment plants (WWTPs) with lower organic loading rates, wherein the need for a long anaerobic feed step may not be as critical as that needed for plants with high organic/nutrient loads. An example of such a plant is the Port Pirie WWTP in regional South Australia. Here the SBRs are hydraulically overloaded due to significant groundwater infiltration into an ageing sewer network, such that the plant operates under relatively low organic loads. Given the hydraulic overloading, the Port Pirie SBRs are currently in need of a hydraulic capacity upgrade, something that may alternatively be achieved through AGS operation to shorten overall cycle times. Due to existing infrastructure limitations, the use of a simultaneous anaerobic feed–decant is difficult to achieve and was considered impracticable. Therefore, it was postulated that the use of a split An–Aer feed to condense the anaerobic feed duration, may be sufficient to reduce the overall cycling time and therefore increase the hydraulic capacity, while still enabling effective AGS establishment and treatment process performance (Figure 1). Accordingly, this study sought to compare the impact of a split An–Aer feed strategy on the ability to establish and maintain mature AGS where there may be limitations in organic loads. Pilot trials were undertaken to compare AGS start-up and performance when employing a dedicated anaerobic feed versus the use of split An–Aer feed under lower organic loads comparable to Port Pirie's full-scale SBR. The impacts of these feeding strategies on SBR performance in terms of nitrogen removal and microbial ecology were also investigated.

2. Methods

2.1 Granular sludge pilot details

A pilot SBR facility was constructed and seeded with conventional activated sludge (CAS) floc (Figure 2(A)). Pilot trials were operated at the Bolivar high salinity WWTP, which has similar sewage characteristics to the Port Pirie WWTP, with elevated salinity in the range of 5–7 g/L total dissolved solids. The pilot AGS SBR facility used here was previously described in detail by van den Akker et al. (2015). In brief, cycle times were controlled using a programmable logic controller (PLC, Figure 2(B)) which mimicked the conditions of the conventional SBR and those needed for AGS development. To investigate the importance of the anaerobic feed, a comparison between a full anaerobic feed (Trial A) and split An–Aer feed (Trial B) was undertaken. For Trial B, it was estimated that 10–20% of the settled biomass bed was exposed to the anaerobic feast conditions (sewage) during the plug feed phase in each cycle, compared to 100% for Trial A. The total duration of aeration in both Trials was 120 minutes and for Trial B, a portion of the aeration phase was coupled with the feed phase (Figure 1). Settling times for Trials A and B were independently adjusted (typically between 5–20 minutes) in order to retain biomass and allow washout of poor settling biomass, and therefore average settling times were 8 and 15 minutes respectively. Trials A and B were conducted consecutively, each having slightly different organic (chemical oxygen demand; COD) loadings at 1.15 and 0.76 kg COD/m^3/d respectively. These reflect typical organic loadings at WWTPs and also the loading experienced at Port Pirie WWTP (Table 1). Organic loading rates in the pilot reactors were varied by changing SBR volumetric exchange ratio.
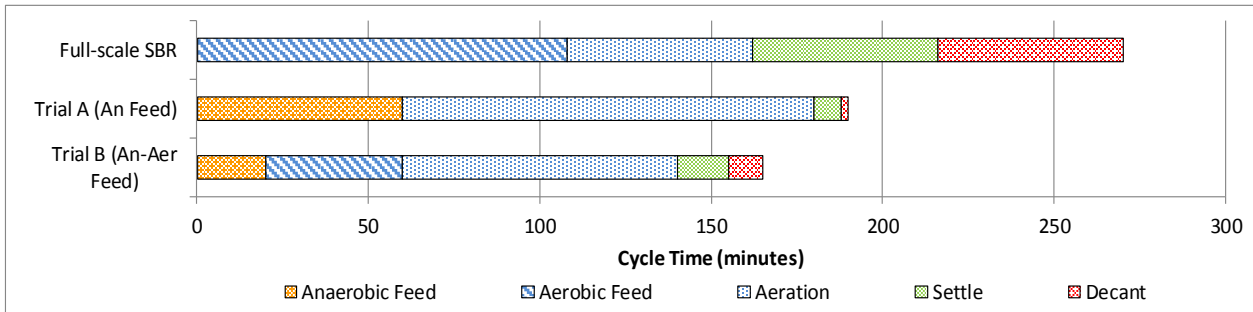
Figure 1: Cycling times for Trial A (anaerobic feed), Trial B (anaerobic–aerobic feed) and full-scale Adelaide metropolitan SBR WWTP.

Table 1: Typical SBR operating organic load and cycle times (minutes)

	COD loading (kg/m^3/day)	Anaerobic feed (min)	Aerobic feed (min)	Aeration (min)	Settling (min)	Decant (min)	Total cycle time (min)
Full-scale CAS SBR (Bolivar WWTP)	0.80	0	108	54	54	54	270
Trial A: anaerobic feed	1.15	60	0	120	8	2	190
Trial B: split An–Aer feed	0.76	20	40	80	15	10	165
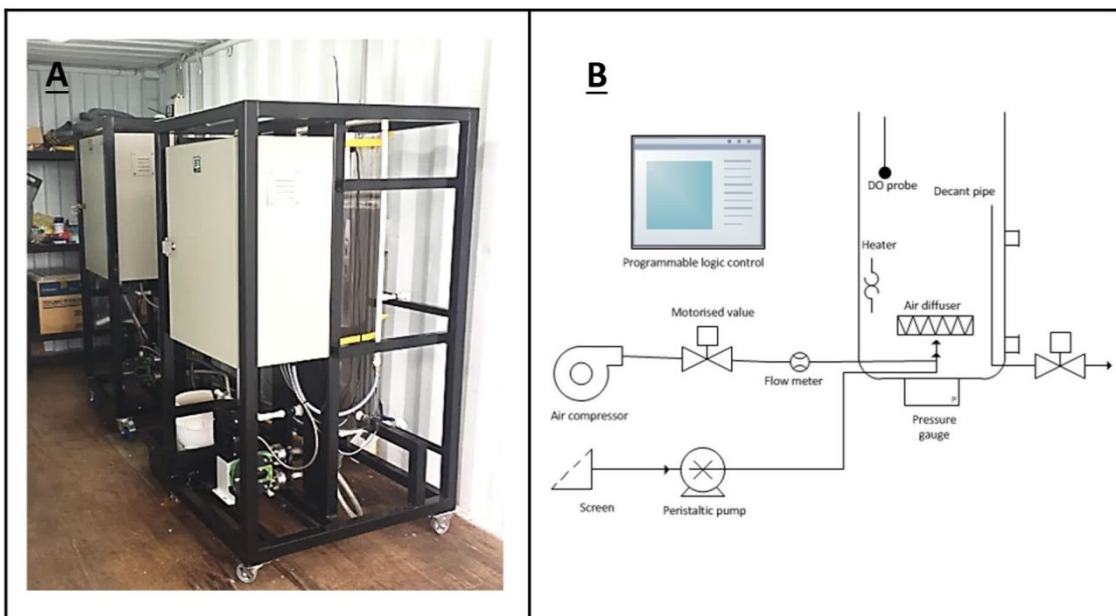
Figure 2: (A) both AGS pilot plants located within a container alongside the full-scale high salinity SBR WWTP, (B) schematic of the SBR pilot plants operated for AGS

2.2 Monitoring and analysis

SBR performance for each trial was monitored over 95 days by measuring temporal changes in concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^--P and COD within the feed sewage and effluent using commercial HACH® test kits (10031, 10019, 10020, 8048 and 8000 respectively). Furthermore, mixed liquor was also analysed for suspended solids (MLSS) and sludge settleability using a 30-minute sludge volume index (SVI_{30}) following standard methods (APHA, 1998). The pilot SBRs were operated alongside a CAS full-scale SBR for comparison (Table 1). Morphological biomass changes were observed via light microscopy (Nikon (SMZ1000) and images were captured using Nikon Digital Sight (DS-U2, Japan) and NIS-Elements D 3.0 (Laboratory Imaging s.r.o.).

2.3 Real-time polymerase chain reaction (qPCR)

Biomass samples were collected from the full-scale CAS SBR and during each of the granular sludge Trials and stored at −80°C for molecular analyses. Duplicate biomass samples were washed three times in phosphate buffer saline (0.2 M) and total genomic DNA was extracted (≈0.25 g biomass) using PowerLyzer® PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, CA) according to manufacturer's protocols. DNA concentration was measured using a Nanodrop 2000C spectrophotometer (Thermo Scientific, Delaware, USA). The abundance of ammonia-oxidsing archaea/bacteria (AOA/AOB), nitrite-oxidsing bacteria (NOB) and denitrifying bacteria were quantified using qPCR targeting 16S rRNA/functional gene primer sets as per Reeve et al. (2016). Analysis was carried out in duplicate using a Rotor-Gene 3000 (Corbett Research, Sydney, Australia). Each 25 μL reaction contained 4 mM $MgCl_2$ (Invitrogen, Carlsbad, CA, USA), 5 μM of oligonucleotide primers (Geneworks, Adelaide, Australia), 0.2 mM dNTPs (Promega, Madison, WI, USA), 1× GoTaq PCR buffer (Invitrogen), 1 U of GoTaq (Invitrogen) and 2 μM SYTO9 (Invitrogen). Thermal cycling conditions involved a primary denaturation at 95°C for 6 min, followed by 55 cycles at 95°C for 20 seconds, 52–66°C for 30 seconds and 72°C for 30 seconds.

2.4 Whole-of-community 16S rRNA fingerprinting

Next-generation sequencing (NGS) was performed on the full-scale CAS floc and mature AGS samples collected from Trial A and B on days 90 and 95 respectively. DNA was extracted using 16S rRNA gene specific primers targeting the region 341F to 806R, using the same extraction method described above. DNA extracts were sent to the Australian Genomic Research Facility (Brisbane, Australia) and next-generation DNA sequencing was performed using a MiSeq sequencer. Sequences were annotated and processed as described in detail by Sawade et al. (2016).

2.5 Data analyses

NGS operational taxonomic unit (OTU) data was presented using Krona interactive visualization program offered by MG-RAST (Ondov et al., 2011). Similarity of the NGS OTU data measured from the AGS and CAS samples was tested via Bray–Curtis similarity analyses using PRIMER 6 (Primer-E, Plymouth, UK) at the phylum and class taxonomic ranks.

2.6 Statistical Analysis

Differences in AGS nutrient removal performance were examined by t-test. Statistical significance was accepted at the $p<0.05$ level. All analyses were achieved using Graphpag PRISM 6 (Version 6.07, Graphpad software, California, USA)

Results and Discussion

3.1 Pilot start-up

Both pilot Trials were initially seeded with 2–3 g/L MLSS taken from the neighbouring aerobically-fed full-scale SBR at the Bolivar high salinity WWTP. Figure 3 compares the SVI_{30} performance of both AGS pilot studies and the full-scale SBR in terms of percent change in SVI_{30} from day zero. This relative comparison approach was adopted to better enable direct comparisons in settling performance given the starting SVI_{30}

values varied between all systems. Both Trials A and B showed significant improvement in biomass settleability within 26 and 44 days, whereby respective SVI_{30} values reached 37.5 and 76.4 mL/g MLSS. This observation corresponded with increased biomass concentration in both pilot systems due to the development of AGS, with MLSS increasing from around 3 g/L initially, to around 5–7 g MLSS/L during the latter stages of both Trials. In contrast to both pilot AGS Trials, SVI_{30} of mixed liquor from the full-scale SBR underwent little change during the same period (Figure 3). AGS development for both Trials was confirmed by analysis of the SVI 5 min/30 min settling performance which showed values of 1.1 for Trial A and 1.2 for Trial B, confirming granular sludge was successfully achieved (Liu et al. (2010)). These results highlight that AGS can be successfully developed and maintained despite differences in organic loading conditions and the use of a split An–Aer feed.
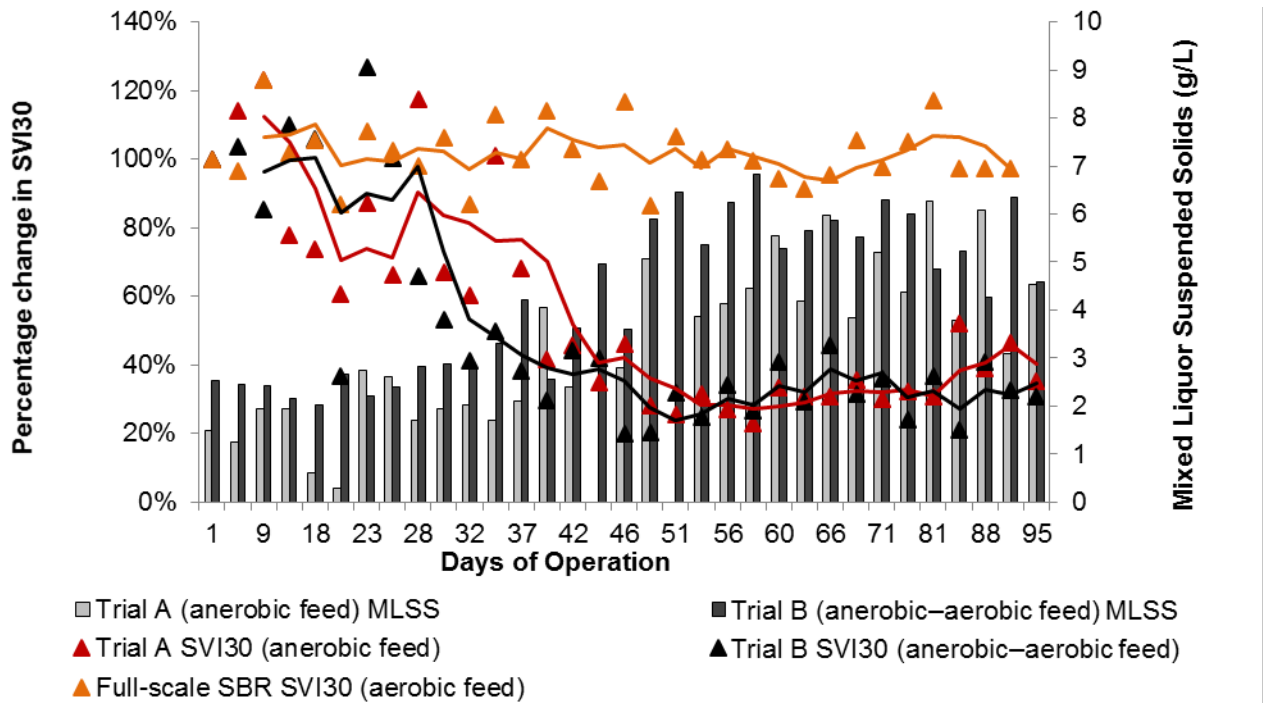
Figure 3: Pilot plant biomass settleability (SVI_{30}) dynamics relative to day zero and mixed liquor suspended solids (MLSS) concentration over time. For comparison, the full-scale SBR SVI_{30} dynamics are provided in orange, again relative to day zero.

3.2 Biomass morphology

MLSS from both Trials and the neighbouring full-scale SBR were viewed using light microscopy to assess morphological changes in reactor biomass (Figure 4). As expected, CAS taken from the full-scale SBR plant appeared as loose, floc-like structures with no clear aggregation (Figure 4A). Granular structures were clearly identified in both samples after 42 days of operation, corresponding to the decrease in SVI_{30}. Filaments were identified protruding from the loose structures. Granules from Trial A (Figure 4B) were irregular in shape, had minimal filamentous protrusions, darker centres and a transparent layer which encapsulated the granules. Granules in Trial B (Figure 4C) were noticeably smoother and more regular in structure, with fewer finger-like projections from the surface and similar to Trial A had a darker core indicating a dense structure. The smoother appearance of AGS morphology from Trial B may be attributed to differences in AGS loading as well as sludge age, as biomass wasting was performed only during Trial B to maintain younger AGS. The irregular granule shape for Trial A may, therefore, be a reflection of an older AGS biomass.
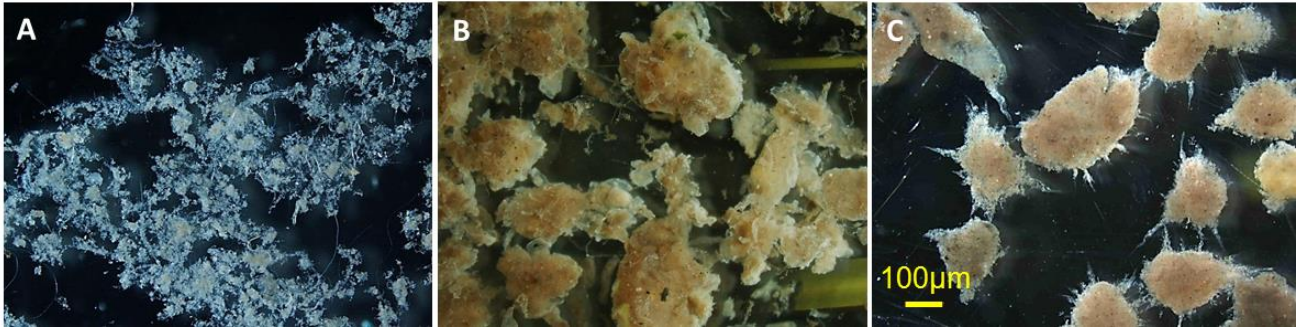
Figure 4: Morphological changes in biomass morphology showing: (A) seeded MLSS floc viewed on day 1 of start-up with a sludge age of 25 days; (B) AGS from Trial A which used a dedicated anaerobic feed taken a sludge age of 28 days; and (C) AGS from Trial B which used a split An–Aer *feed taken at a sludge age of 22 days. All images taken at 10× magnification.*

Interestingly, our observations are in contrast to those of McSwain et al. (2004) who identified that granules fed with a dedicated anaerobic feed with no biomass mixing were more regular in shape, denser and had fewer filamentous protrusions, while granules fed in split An-Aer fashion were less dense and more irregular in shape and had relatively more filamentous protrusions. Whilst the observations of McSwain and colleagues were in apparent contradiction to observations of granular morphology during our study, the differences in loading, operation and substrate may help explain these differences. For example, the reactors of McSwain et al. (2004) were fed with a synthetic substrate supply (glucose + peptone) at 2.4 kg COD/m^3/d which was some two- to three-fold higher than the organic loading rate of Trials A and B (1.15 and 0.76 kg COD/m^3/d) and the full-scale SBR (0.8 kg COD/m^3/d) respectively. The higher organic loading rate of McSwain et al. (2004) would be expected to facilitate filamentous bacteria growth over granule-forming organisms, resulting in the more 'fluffy' appearance the authors observed.

The irregular, finger-like protrusions extending from the granules seen during Trial A may have been a result of the higher organic loading that can lead to the development of steep substrate and oxygen diffusion limitation gradients, which has been shown to induce filamentous outgrowth and a 'fluffy' exterior. This was identified in modelling work by Picioreanu et al. (1998) who found that when the ratio of biomass growth rate versus diffusive transport is high, sharp gradients existed that resulted in "finger-like" or "mushroom" biofilm outgrowths. Experimental work conducted later by Mosquera-Corral et al. (2005) made similar observations, where sharp concentration gradients and diffusion limitations, induced by a decrease in dissolved oxygen concentrations, had resulted in the development of irregular, external floc-like structures on AGS. The more regular appearance and 'smooth' surface of granules such as that observed in Trial B here is said to develop when the ratio of biomass growth versus diffusive transport is low (i.e. flatter oxygen or substrate gradients) (Picioreanu et al., 1998, Pronk et al., 2015a). For highly loaded AGS systems, this requires the use of a slow anaerobic feed to ensure even biomass production throughout the granule, which is important for granule stability (Pronk et al., 2015a). For our study, it was likely that the decrease in the organic load during Trial B had similar affect in reducing the ratio of biomass growth rate versus substrate transport ratio, and hence a long anaerobic feed was less crucial.

3.3 AGS performance

SBR performance data for the pilot Trials are summarised in Table 2. Whilst there was a clear change in the morphology of the mixed liquor in both AGS Trials, the nitrification performance of the pilot SBRs remained quite stable for the entire duration, with ammonium removal typically >95%. Total nitrogen removal was initially more variable when compared to the CAS, which was attributed to periods of over aeration within the pilot AGS systems, which resulted in reduced denitrification performance and NO_3^--N accumulation. Total nitrogen removal did however improve during both Trials A and B from 61% and 41% after the first 40–45 days respectively, to 97% and 90% as the AGS matured (Figure 5). This improvement was most likely due to the development of anoxic denitrifying cores within the granules which facilitated simultaneous nitrification–

denitrification (de Kreuk et al., 2005a). t-test comparing the AGS performance of Trial A and B revealed no significant difference in the removal of COD ($P = 0.092$), TN ($P = 0.28$) and ammonium ($P = 0.157$).

Table 2: Pilot granular sludge SBR and full-scale SBR performance data during the 95 day study (data shows minimum and maximum performance range for each parameter)

	Trial A (anaerobic feed)	Trial B (anaerobic–aerobic feed)	Full-scale SBR (aerobic feed)
Organic load (kg COD/m^3/d)	1.15	0.76	0.80
SVI_{30}	37–188	58–131	211–360
MLSS (g/L)	1.7 – 6.3	2.1 – 6.8	2.5–3.5
Sludge age (days)	14–34	15–31	21–25
Aeration DO (mg O_2/L)	1.0–3.0	1.0–3.5	1.0–3.0

The dissolved oxygen concentration range was optimised during the study to be between 1.0–3.5 mg/L (regulated via PLC control), which also assisted total nitrogen removal performance (Table 2).

Soluble phosphorus (PO_4^{3-}) removal was lower in Trial B than in Trial A (t-test, $p = 0.009$). PO_4^{3-} Removals in Trial A ranged between 5.4 and 49.7 %, and for Trial B 4.3 and 17.3 % mg/L (Figure 5). PO_4^{3-} concertation profile taken during the cycle also showed reduce PAO activity in Trial B, as seen by the limited release of PO_4^{3-} during the anaerobic feed and subsequent uptake during aeration (Supplementary Information S1). Net phosphate removals seen in both Trials were notably reduced than compared to de Kreuk et al. (2005a), who reported on average 94% PO_4^{3-} removal efficiency in small laboratory-scale AGS reactors operated under comparable DO (1.8 mg O_2/L) and organic loadings (1.6 kg COD/m^3/d). The lower PO_4^{3-} removal performance here is most likely due to the high saline nature of our sewage, as salinity is known to inhibit PAOs (Corsino et al., 2016, Welles et al., 2014). For example, Pronk et al. (2013) showed that nitrite (NO_2^{-}) levels above 4.0 mg/L in addition to high salinity (>200 mg/L) were inhibitory to PAOs, with evidence that salinity levels of 20 g/L in the absence of NO_2^{-} also yielded reduced PAO activity. Similarly, Bassin et al. (2011) showed that increased salinity coupled with high NO_2^{-} concentrations resulted in a loss of *Nitrospira* and PAOs. The poor biological PO_4^{3-} removal performance observed during Trial B in particular can be explained by the lower COD loading rate during this Trial and marked reduction in anaerobic feed duration (Table 1). Such conditions would be expected to be unfavourable to PAOs (Pronk et al., 2013, de Kreuk and van Loosdrecht, 2004, de Kreuk et al., 2007).
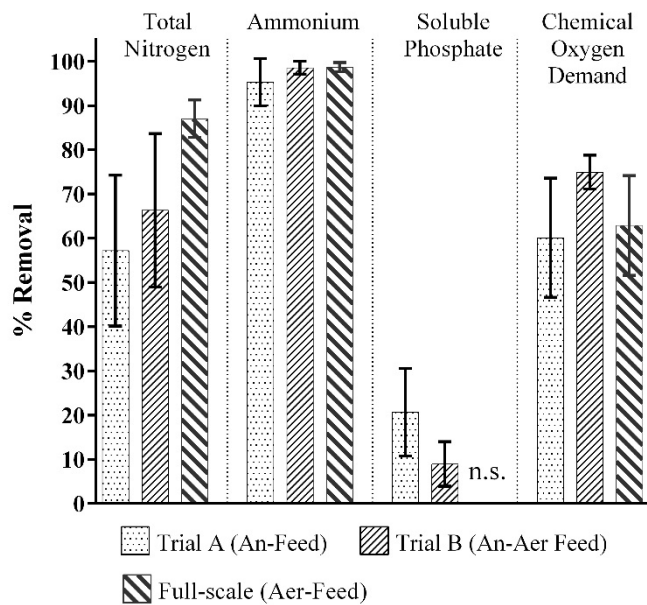
Figure 5: Comparative treatment performance data (100−($C_{effluent}$/$C_{influent}$)×100) for Trial A and B versus the full-scale SBR over the 95 day period (data shows mean ± 1 S.D. for each nutrient measured).

3.4 Microbial ecology of key nitrogen cycling organisms

Microbiological diversity of CAS flocs sampled from the full-scale SBR and mature AGS sampled from Trials A and B were analysed using qPCR to compare the abundance of key nitrifying and denitrifying microorganisms. Notable differences were seen in the abundance of ammonia-oxidsing bacteria (AOB) and ammonia-oxidsing archaea (AOA) in particular based on measured target gene copy numbers (Figure 6). AOB were 4- and 2-$\log_{10}$ more abundant in the full-scale CAS flocs compared to AGS sampled during Trials A and B respectively. In contrast, AOA were markedly more abundant in AGS than CAS flocs by some 5- and 7-$\log_{10}$ orders for Trials A and B respectively. The preference of AOA for AGS may be attributed to the longer sludge age (AOA are generally slower growing than AOB), as well as the presence of potentially steep oxygen and nutrient gradients that are likely to exist throughout the granule depth. These factors have been identified as likely environmental drivers for niche differentiation and promotion of AOA over AOB in activated sludge-based wastewater treatment systems elsewhere (Short et al., 2013).
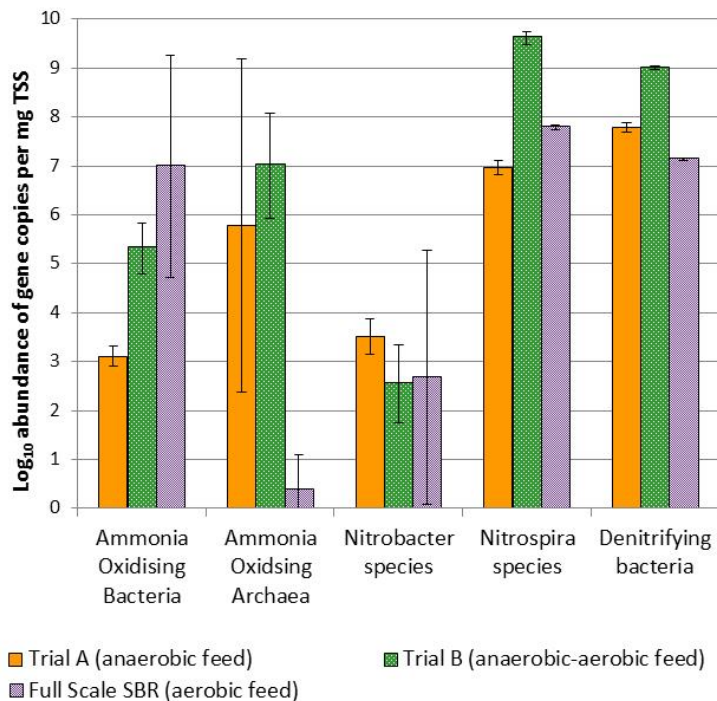
Figure 6: Microbial populations for AGS Trials A (anaerobic feed) and B (split An–Aer feed) and full-scale (aerobic feed) conventional SBR, comparing the abundances of ammonia-oxidising bacteria (AOB), ammonia-oxidising archaea (AOA), Nitrobacter and Nitrospira lineages and denitrifying bacteria. Error bars represents ± 1 standard deviation of log_{10}-transformed data.

Nitrospira spp. was clearly the dominant NOB over *Nitrobacter* spp. in both the full-scale CAS and AGS pilot SBRs (by some 3–6-log_{10} orders), with no clear differences in relative *Nitrospira* and *Nitrobacter* abundance between CAS and AGS configurations (Figure 6). Itoi et al. (2007) showed that in mature biofilms, *Nitrospira* was present only in the surface of the biofilms and not present in deeper layers. As AGS is essentially a biofilm without a support media, the presence of *Nitrospira* in the surface layers may also be expected here; although the spatial distribution of NOB in our AGS was not assessed. Furthermore, *Nitrospira* can out-compete *Nitrobacter* at low NO_2^- concentrations (Blackburne et al., 2007). While only effluent NO_2^- data was collected during Trial A (0.9–9.8 mg/L), data from Trial B showed mixed liquor NO_2^- concentrations of 1–7 mg/L and just 0–1.6 mg/L in the effluent. The lower effluent NO_2^- concentrations during Trial B may have favoured *Nitrospira* over *Nitrobacter* as seen in Figure 6; although we acknowledge that bulk measurements may not necessarily reflect the NO_2^- concentrations that NOB would be exposed to within the granules.

Regarding the abundance ratios of NOB/AOB, these were 1,000–3,000-fold higher for the AGS relative to the CAS biomass. This apparent disproportion of the NOB/AOB ratio seen in AGS may also relate to the presence of a 'nitrite loop' within AGS, wherein denitrifiers supply NOBs with additional NO_2^- from the reduction of nitrate. This observation is supported by the findings of Winkler et al. (2012) who also reported higher NOB/AOB ratios in AGS than floc-based CAS. For denitrifying bacteria, results showed notionally higher abundance in AGS relative to CAS biomass (<1-log_{10}). While not strikingly different, higher denitrifier abundance in AGS over CAS could possibly relate to the development of an anoxic core within the granule (de Kreuk et al., 2005a).

3.5 Community diversity profiling

Microbial community fingerprinting was conducted on DNA extracted from AGS and CAS flocs using 16S rRNA gene-based primers as per Section 2.4. The taxonomic distribution of these reuslts are presented in Figure 7 (A-C). An interactive version of Figure 7 is available in the Supplementary Information (S2-S4). Bray–Curtis similarity analysis (Supplementary Information S5), showed high levels of similarity between

AGS communities from both Trials A and B at the phylum and class taxonomic level, suggesting that the different AGS feed strategies and loadings had no measurable impact on microbial ecology at these taxonomic levels. A greater divergence in similarity can be seen (Figure S4) when comparing the ecology of AGS and CAS samples taken from the neighbouring full-scale SBR (i.e. pilot SBR seed sludge).
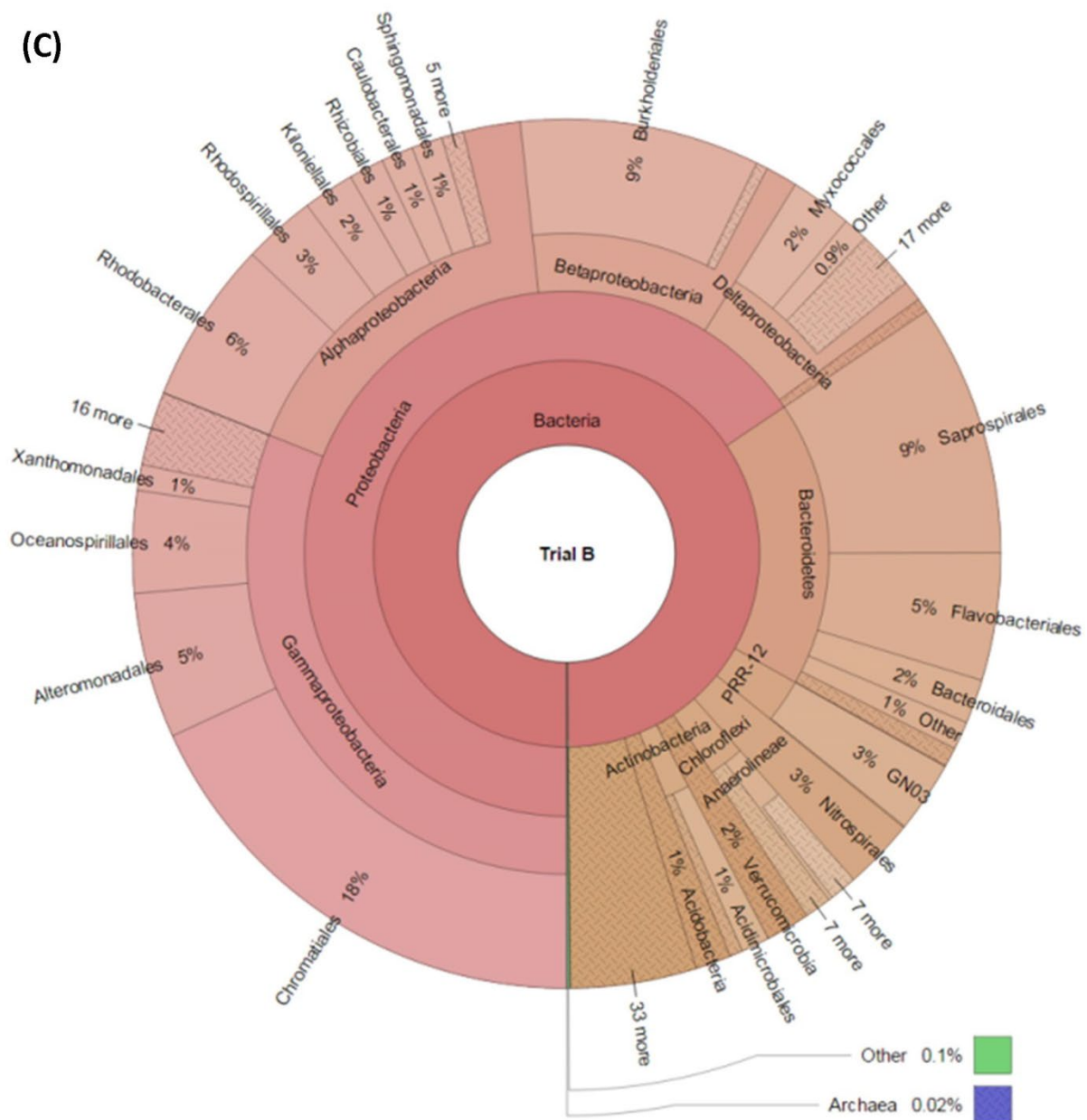

(A)

(B)
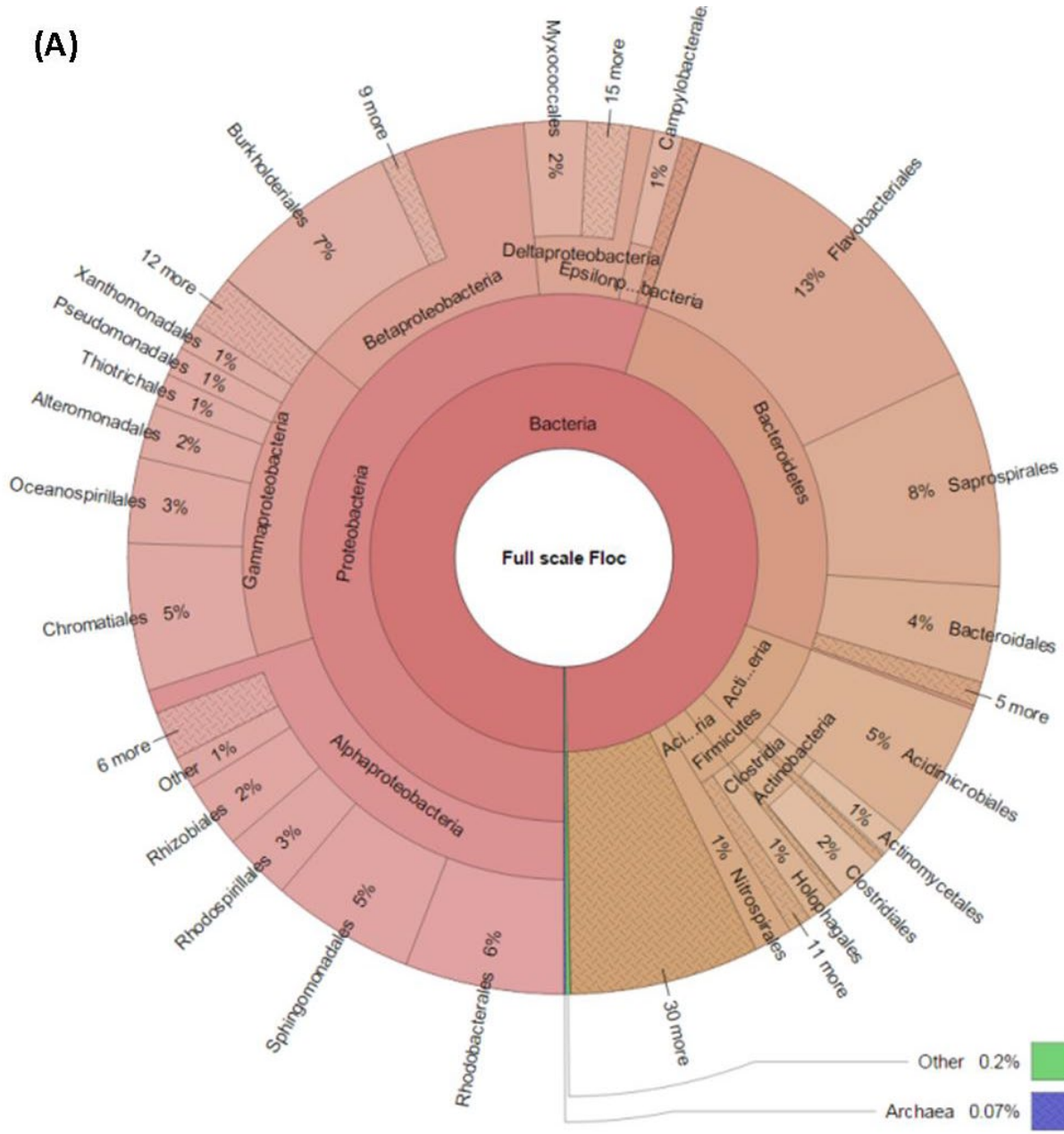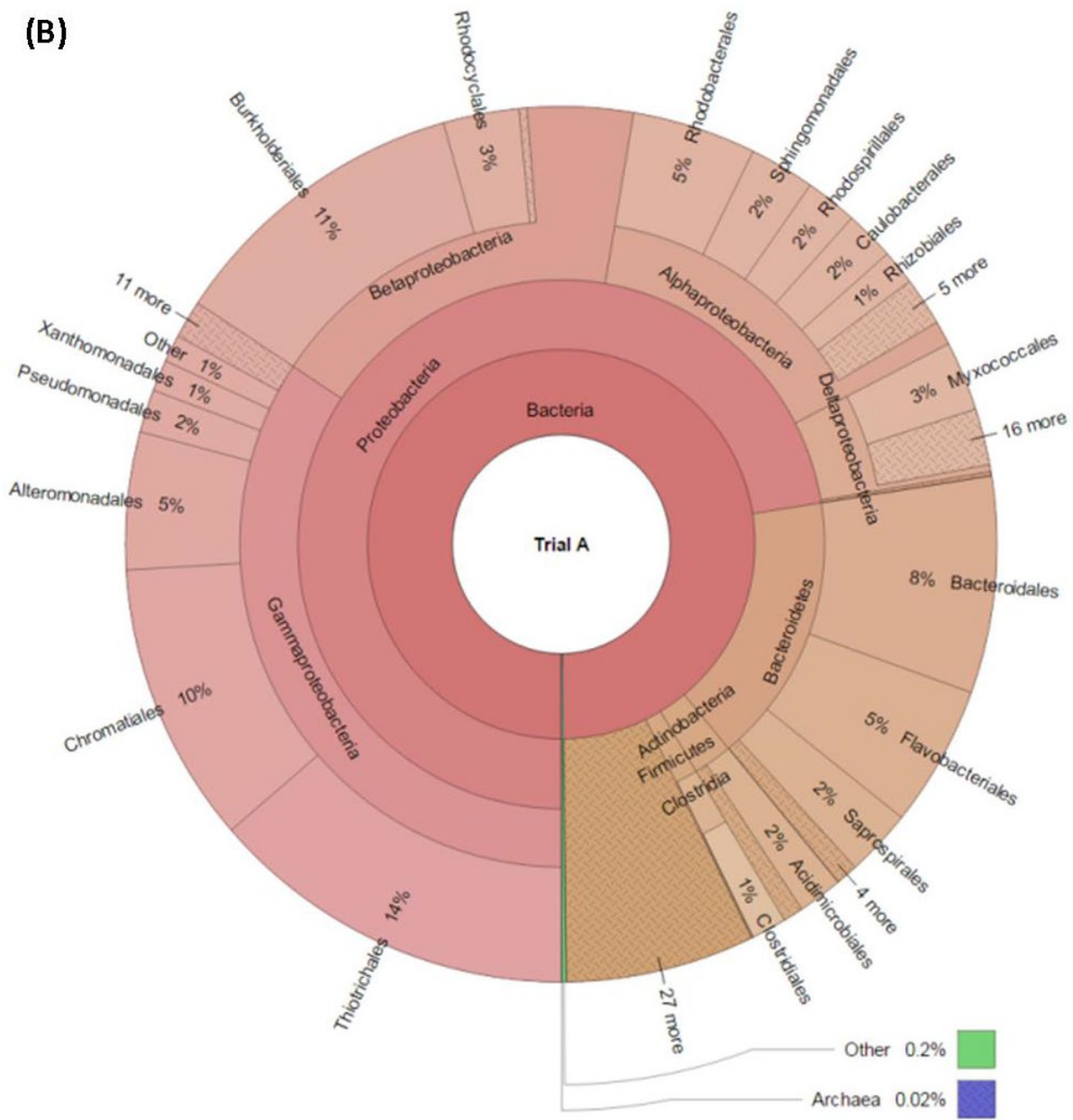
Figure 7: Taxonomic distribution of the microbial community from the 16S rRNA gene-based community sequencing data for: (A) full-scale (aerobic feed) SBR floc; (B) Trial A (full anaerobic feed); (C) Trial B (split An–Aer feed). Figure prepared by Krona interactive visualisation program. Interactive versions provided in Supplementary Information S1, S2 and S3 respectively.

In all three samples, Bacteria were the predominant domain, with Archaea representing <0.1% of total community relative abundance (Figure 7). Within the detected Archaea sequences, phylum Thaumarchaeota, which contains all known AOA (Gao et al., 2013), was not detected despite AOA being detected by qPCR and outnumbering AOB in both AGS samples (Figure 6). The discrepancy is likely due to the changes in primers used for detection for qPCR and NGS (i.e. the quantitative PCR conducted targets the archaeal *amoA* gene, while the NGS targets the 16S rRNA gene). As the qPCR targets a specific gene, this gives the abundance of the gene in each sample; NGS conversely only yields relative abundance data in whole-of-community terms, such that AOA may be relatively underrepresented among the entire community diversity, of which ammonia-oxidisers are only a small constituent. Of the Bacteria, Proteobacteria were the

dominant phylum in all three samples (Figure 8), representing 55–73% of the total bacterial community, followed by Bacteroidetes (17–26%). When comparing AGS with CAS, Proteobacteria were the key divergent class, varying by 10% (Figure 8 and S4). Within Proteobacteria, Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria predominated in all samples, but their relative abundances varied. In the CAS floc sample (Figure 8), Alphaproteobacteria accounted for the largest portion of phylum Proteobacteria organisms, whereas in the AGS biomass (Trial A and B), Gammaproteobacteria was the dominant class, representing 47–50% in the AGS Trials. This enrichment of class Gammaproteobacteria microbes in AGS here is consistent with the known functional ecology of AGS, as GAOs are well represented within this class (Lemaire et al., 2008, Seviour and Nielsen, 2010).
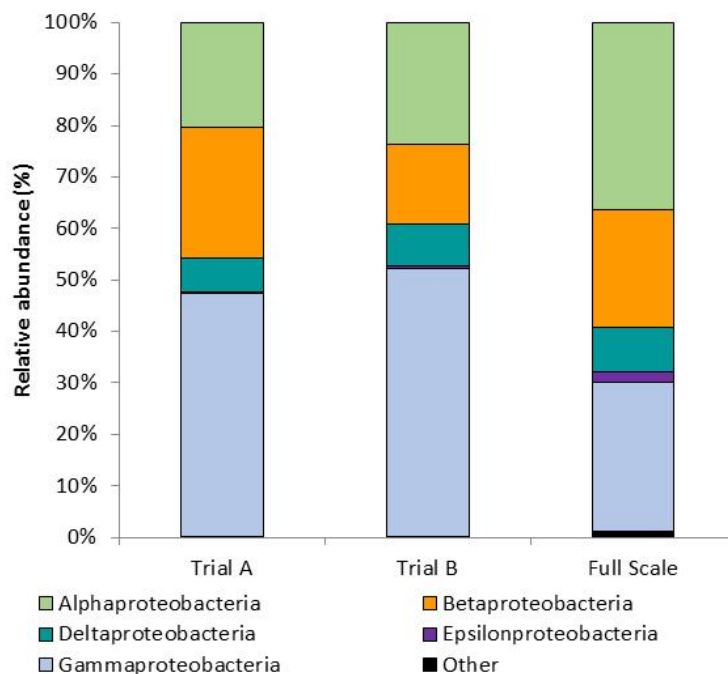
Figure 8: Relative abundance of phylum Proteobacteria organisms in AGS pilot Trials A (anaerobic feed) and B (An-Aer feed) versus the full-scale SBR CAS floc. Data relates to samples taken for Trial A and B on day 90 and 95 respectively.

Of further interest is the change in Betaproteobacteria abundance between the three samples. Based on relative abundance, the Betaproteobacteria fraction of Proteobacteria was higher in Trial A and the CAS floc compared to Trial B. Notably, Betaproteobacteria includes the putative PAO *Candidatus* 'Accumulibacter phosphatis' which has been shown to be critical for PO_4^{3-} uptake in aerobic granular sludge (Bassin et al., 2012). The relative abundance of this organism alone changed from 1.3% in Trial A, to <0.1% in Trial B and 0.2% in the full-scale CAS SBR, complementing the reduced biological PO_4^{3-} removal performance (Figure 5) and PAO activity (S1) observed during Trial B. Interestingly, *Candidatus* 'Accumulibacter phosphatis' is known to be favoured by anaerobic/aerobic cycling conditions in particular, so it seems likely that the extended anaerobic feed conditions (Cydzik-Kwiatkowska and Zielińska 2016) of Trial A promoted the selective development of this organism over the split An–Aer feed conditions of Trial B which would have yielded relatively more anoxic/aerobic conditions during the feed phase. Phosphate removal in Trial A and B was also lower than expected for typical AGS systems based on typical removal performance observed in similar AGS systems elsewhere (de Kreuk et al., 2005b, de Kreuk et al., 2007, Pronk et al., 2013). This observation, together with the observed enrichment of Gammaproteobacteria, suggests that GAOs were likely to have dominated over PAOs in our AGS system. Trial B also had increased abundance of Nitrospirales (3% of total Bacteria) compared to Trial A and full-scale (0.1 and 1% respectively) which was also confirmed by qPCR results (Figure 6).

4. Conclusion

Pilot-scale research here showed that the formation and stability of AGS may not be as dependant on a long anaerobic feed as previously published research has indicated. The start-up and performance of AGS when using a split An–Aer feed under low organic loads (0.76 kgCOD/m^3/d) was comparable to the use of a dedicated anaerobic feed under typical higher organic loading (1.15 kgCOD/m^3/d). Additionally, we have shown that different feed strategies (anaerobic versus split aerobic–anaerobic) and organic load, had a relatively minor impact on AGS ecology at a higher order taxonomic levels (phylum and class) and more importantly, had little impact on the functional microbial ecology and treatment performance of the AGS systems. We also showed that there was a shift in diversity of these organisms between CAS and AGS, which was related to the change in biomass structure. In particular NGS data showed that there were changes within the Proteobacteria class, which showed reduced abundance in PAOs in Trial B in relation to GAOs. From an engineering standpoint, we have shown that a dedicated anaerobic feed is not critical to achieve AGS, thus potentially increasing the scope for SBR reactor retrofitting and reducing the associated retrofitting costs for transforming CAS SBRs to AGS SBRs for shorter operating cycles and increased hydraulic capacity of existing plants.

5. Acknowledgements

**Supplementary Information (S1).** PO_4^{3-} removal profile for Trial A and B.
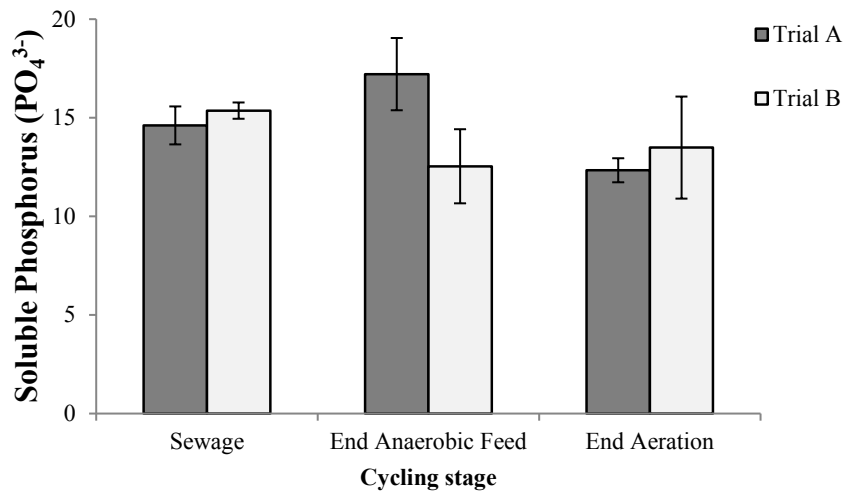
Supplementary Information (S2). Interactive graph showing the taxonomic distribution of the microbia community from the 16S rRNA gene-based community sequencing data for the full-scale (aerobic feed) SBR CAS floc.

Supplementary Information (S3). Interactive graph showing the taxonomic distribution of the microbial community from the 16S rRNA gene-based community sequencing data for Trial A (full anaerobic feed) AGS.

Supplementary Information (S4). Interactive graph showing the taxonomic distribution of the microbial community from the 16S rRNA gene-based community sequencing data for Trial B (split An–Aer feed).

Supplementary Information (S5): Cluster plots showing Bray–Curtis similarities between full-scale CAS and AGS (Trial A and B) at the Phylum level (A), Class level (B) and comparison of the Proteobacteria Class (C). Similarity coefficient of 100 means two samples share identical species distributions and a coefficient of zero means they share no common species.
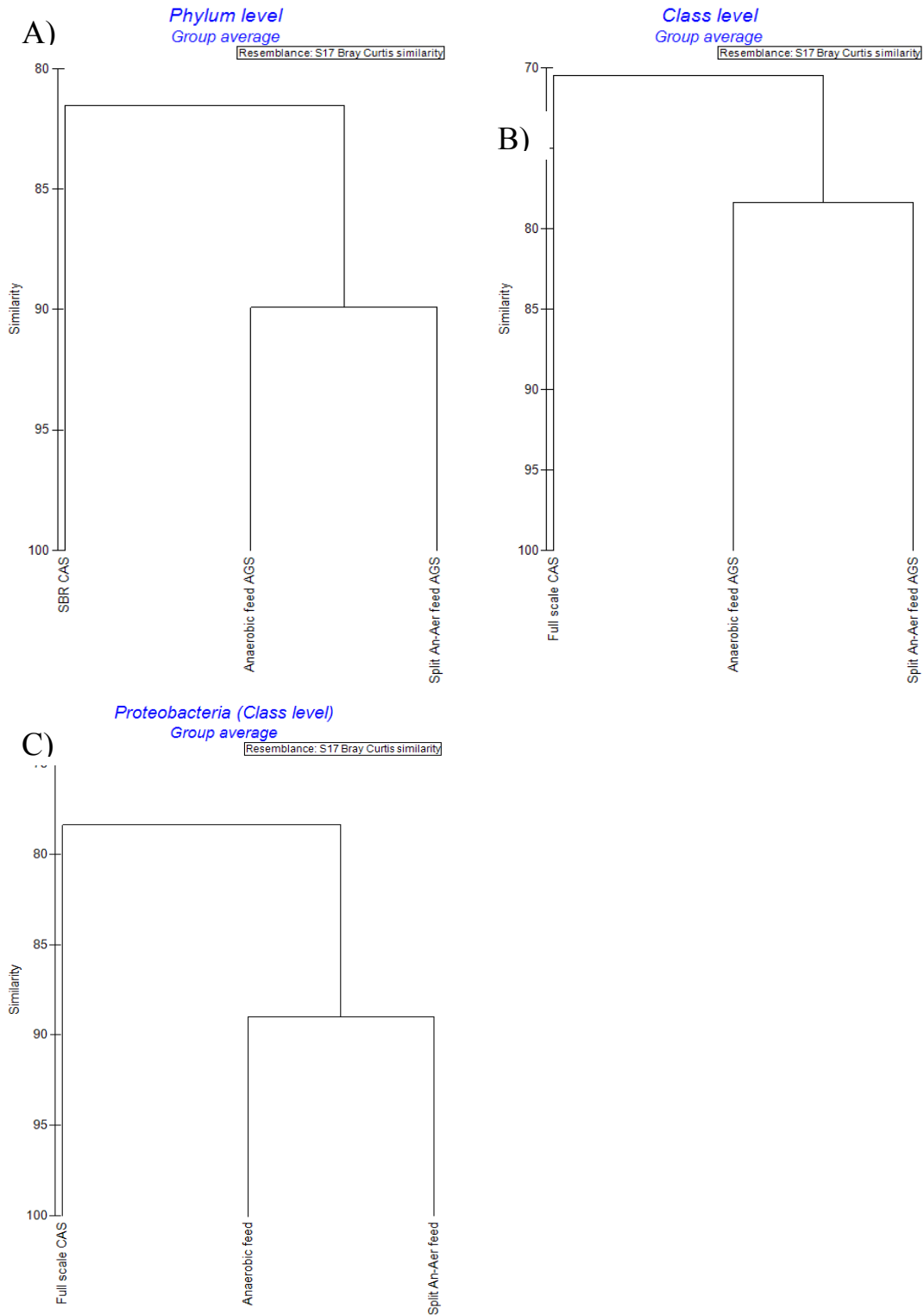
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Appendix D. Research output 6: Understanding the role of wastewater feeding strategy (anaerobic or split anaerobic–aerobic) on AGS development and functional performance

This research output has been published in the following:

Title: Ecology and performance of aerobic granular sludge treating high-saline municipal wastewater

Authors: Benjamin J. Thwaites, Ben van den Akker, Petra J. Reeve, Michael D. Short, Nirmala Dinesh, Juan Pablo Alvarez-Gaitan, Richard Stuetz

Abstract: The successful development of Aerobic Granular Sludge (AGS) for secondary wastewater treatment has been linked to a dedicated anaerobic feeding phase, which enables key microbes such as poly-phosphate accumulating organisms (PAO) and glycogen accumulating organisms (GAO) to gain a competitive advantage over floc-forming organisms. The application of AGS to treat high-saline sewage and its subsequent impacts on microbial ecology, however, is less well understood. In this study, the impacts of high-saline sewage on AGS development, performance and ecology were investigated using molecular microbiology methods. Two feeding strategies were compared at pilot scale: a full (100%) anaerobic feed; and a partial (33%) anaerobic feed. The results were compared to a neighbouring full-scale conventional activated sludge (CAS) system (100% aerobic). We observed that AGS developed under decreased anaerobic contact showed a comparable formation, stability and nitrogen removal performance to the 100% anaerobically-fed system. Analysis of the microbial ecology showed that the altered anaerobic contact had minimal effect on the abundances of the functional nitrifying and denitrifying bacteria and Archaea; however, there were notable ecological differences when comparing different sized granules. In contrast to previous work, a large enrichment in PAO in AGS was not observed in high-saline wastewater, which coincided with poor observed phosphate removal performance. Instead, AGS exhibited a substantial enrichment in sulfide-oxidising bacteria (SOB), which was complemented by elemental analysis that identified the presence of elemental sulfur precipitation. The potential role for these organisms in AGS treating high-saline wastewater is discussed.

INTRODUCTION

Aerobic granular sludge (AGS) has been shown to be a viable option for various municipal wastewater treatment applications, with recognised advantages such as increased hydraulic capacity and reduced physical foot print. AGS is commonly achieved using sequencing batch reactors (SBRs), with the technology utilising rapid-settling, dense microbial granules in place of floc-based conventional activated sludge (CAS). The conversion process has been shown to be highly dependent on the implementation of a dedicated anaerobic feed and short sludge settling times. The anaerobic feed selects for slow-growing microbes such as polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs), which have been shown to play a critical role in granule development. These microbes have been identified as being able to store the bioavailable organic carbon generated during the anaerobic feed (de Kreuk and van Loosdrecht, 2004), with PAOs also playing a role in the removal of phosphate (PO_4) (Bassin et al., 2012). These groups of bacteria are predominantly found within the phylum *Proteobacteria* and class *Deltaproteobacteria* and *Gammaproteobacteria* (Zhang et al., 2011, Diaz et al., 2003).

Whilst AGS technology has been widely researched and applied in many locations worldwide, there is little known about the potential impacts of high-saline municipal wastewater on granule development and treatment performance. High-saline wastewater streams can result from groundwater infiltration into sewer networks (e.g. coastal locations) and often have high concentrations of sulfate and sulfide; this may lead to issues with granule formation and stability due to inhibition of extracellular polymeric substances (EPS) production, nitrite accumulation and PAO inhibition (Welles et al., 2014, Li et al., 2014). Previously, van den Akker et al. (2015) showed that development of AGS was possible in high-saline wastewater when using a dedicated anaerobic feed and long (≥ 2 hours) aerobic phase. Winkler et al. (2012b) showed that granules incubated for a short time in varied salinity concentrations (up to 40 g/L NaCl) had a reduced settling velocity that had the potential to cause biomass washout. However, once the salt concentration equalised there was no effect on settling velocity. This study was based on laboratory-grown granules under relatively short biomass salinity acclimation

periods (up to 24 h). Further work on the impact of varied salinity concentrations on the granulation process was conducted by Li et al. (2017) who found that rapid granulation occurred when operated under the highest percentage (100%) of seawater. The ammonia removal efficiency was initially reduced, however increased to 90% after 140 days of operation. This study also found that the presence of seawater severely reduced the maximum ammonium and nitrite oxidation rates.

To date, most of the published data on salinity impacts in AGS were largely derived from bench-scale reactors fed with synthetic wastewater and under periods of short-term exposure to high salinity conditions. Complementary field based research at larger scale is, therefore, required to validate laboratory findings. Accordingly, this study investigated the impact of high-saline municipal wastewater on AGS formation, stability and microbial ecology at pilot scale. Secondly, the impacts of different feeding strategies were investigated to better understand the influence of anaerobic versus aerobic feed conditions. Characterisation of the AGS community was done based on whole-of-community 16S rRNA profiles and targeted analysis of functional genes specific for nitrifying and denitrifying microorganisms, with AGS microbial ecology compared to a neighbouring full-scale CAS SBR at functional and whole-of-community levels.

METHODOLOGY

The pilot scale SBR was located at a large metropolitan wastewater treatment plant (WWTP) (Adelaide, South Australia), which receives high-saline (6,000–7,000 mg TDS/L) municipal wastewater, a result of high volumes of infiltration into the sewer network. The secondary activated sludge treatment at the full-scale WWTP consists of six SBRs with a design capacity of 32 ML/d. The pilot scale reactor (63.9 L volume) was located within a weather-proof climate controlled container and was controlled using programmable logic controllers allowing cycle times, volumetric exchange and air flow to mimic CAS maintenance or develop AGS (van den Akker et al., 2015). The pilot SBR was fed with screened (2 mm mesh) municipal wastewater (Table 1) sourced from the full-scale WWTP inlet and was inoculated with 3 g/L of flocculent biomass from the neighbouring full-scale CAS reactor.

Table 1: Median concentration of key parameters found in the high-saline municipal sewage (n ≥ 10 ± 1SD).

	Total COD (mg/L)	Ammonia (mg/L)	Total Nitrogen (mg/L)	Sulfate (mg/L)	Conductivity (µScm)	Total dissolved solids (TDS) (mg/L)
High-saline municipal wastewater	534.9 ± 64.7	35.1 ± 3.2	55.8 ± 7.7	668.6 ± 90.8	11393.5 ± 426.2	6535 ± 251.8

The COD loading rates and cycle times that were used in the operation of the AGS pilot and Full-scale SBRs are given in Table 2. For the AGS trials, two feeding strategies were compared at pilot scale: A full 100% anaerobic feed (Strategy A) and a partial 33% anaerobic feed (Strategy B). Strategy A was assessed to investigate the impacts of AGS under high saline conditions using operational parameters analogous to previous AGS studies (Morgenroth et al., 1997, Beun et al., 1999). Strategy B was assessed to understand the impacts of AGS operating under lower COD loads which was comparable to the neighbouring full-scale conventional activated sludge (100% aerobic feed) system (Strategy C). In light of the lower COD loads used in Strategy B, the impact of employing a reduced anaerobic feed duration was investigated as this further reduces the cycle time and makes AGS easier to retrofit within existing SBRs. The performance and stability of the AGS trials was monitored for 95 and 113 days.

Table 2: Operating parameters of the pilot AGS and full-scale CAS SBRs showing organic loading rates and cycle time phases.

	COD Loading (kg/m³/day)	Anaerobic Feed (minutes)	Aerobic Feed (minutes)	Aeration (minutes)	Settling (minutes)	Decant (minutes)	Total Cycle time (minutes)	Trial time (days)
Strategy A (100% Anaerobic)	1.15	60	-	120	8	2	190	113
Strategy B (33% Anaerobic)	0.76	20	40	80	15	10	165	95
Strategy C (100% Aerobic)	0.80	-	54	108	54	54	270	∞

Biomass and Nutrient Analysis

Nitrogen removal was examined throughout the trial periods by measuring COD, PO_4-P, NH_4^+-N, NO_2^--N and NO_3^--N in wastewater, mixed liquor and secondary effluent. Analysis was conducted using HACH colorimetric test kits 8000, 8048, 10031, 10019 and 10020. Suspended solids concentration and morphology was examined twice weekly, sludge settleability was determined using a 30-minute Sludge Volume Index (SVI_{30}) (APHA, 1998). Morphological changes were examined via light microscopy (Nikon (SMZ1000) and images were captured using Nikon Digital Sight (DS-U2, Japan) and NIS-Elements D 3.0 (Laboratory Imaging s.r.o.).

Microbial Ecology Analysis

Biomass samples were collected from each AGS feeding strategy (day 90). An additional sample was collected from the aerobically fed, full-scale CAS SBR. All biomass samples were stored at −80°C prior to preparation for molecular analysis. A biomass sample from Strategy A was size-separated using increasing mesh sieves (300, 1,000, 1,400 μm) with the retained biomass washed off the mesh using sterile tap water. DNA was extracted using PowerLyzer® PowerSoil® DNA Isolation Kit (MOBIO Laboratories Inc., Carlsbad, CA.) with the biomass being washed in sterile phosphate buffer saline (PBS) prior to following the manufactures extraction method. The extracted DNA was quantified using a Nanodrop 2000C spectrophotometer (Thermofischer, Delaware). Analysis was conducted on the nitrogen removal functional gene groups (ammonia-oxidising archaea/bacteria (AOA/AOB), nitrite-oxidising bacteria (NOB) and denitrifying bacteria) using quantitative polymerase chain reaction (qPCR) targeting 16S rRNA/functional gene primer sets by Reeve et al. (2016). The reaction was carried out in duplicate using a Rotor-Gene 3000 (Corbett Research, Sydney, Australia). Each 25 μL reaction contained 4 mM $MgCl_2$ (Invitrogen, Carlsbad, CA, USA), 5 μM of oligonucleotide primers (Geneworks, Adelaide, Australia), 0.2 mM dNTPs (Promega, Madison, WI, USA), 1× GoTaq PCR buffer (Invitrogen), 1 U of GoTaq (Invitrogen) and 2 μM SYTO9 (Invitrogen). Thermal cycling conditions involved a primary denaturation at 95°C for 6 min, followed by 55 cycles at 95°C for 20 seconds, 52–66°C for 30 seconds and 72°C for 30 seconds.

High throughput sequencing was performed on the DNA extracted from the biomass samples collected on day 90. Genomic DNA was extracted using the same extraction method described above. DNA extracts were sent to the Australian Genomic Research Facility (Brisbane, Australia) where analysis was performed on an Illumina MiSeq sequencer using 16S rRNA gene specific primers targeting the region 341F to 806R.

SEM and EDS Analysis

Biomass samples were collected and freeze dried for 72 hours. Samples were then coated with gold to a thickness of 20nm. Scanning electron microscopy (SEM) was conducted using a Zeiss Merlin operated with a working distance of 6.0mm, electron high tension (EHT) of 10.00kV. Samples were further analysed using

energy dispersive x-ray spectroscopy (EDS) detector mounted into the SEM chamber. This allowed for detection and identification of the elemental composition of user-specified field of the sample.

XFM Analysis

Elemental X-ray fluorescence analysis was conducted to investigate granule structure, density and metals concentration using the X-ray fluorescence microscopy (XFM) beamline at the Australian Synchrotron (Paterson et al., 2011). Biomass samples from strategy A were freeze dried and mounted on Kapton tape for analysis. Images were analysed as per the protocol of Donner et al. (2011) with co-localisation determined using tri-colour mapping.

RESULTS and DISCUSSION

Start-up and performance

During start up, there was a large increase in the mixed liquor biomass concentrations during the pilot trials with the final steady-state concentration being 5–7 g/L (Supplementary Figure 1A). The sludge settling performance achieved a SVI_5/SVI_{30} ratio of 1.1 in the full anaerobic feed within 37 days, similarly the settling ratio of the partial anaerobic feed decreased to 1.2 within the initial 51 days of operation; this decreased ratio was consistent with previous findings by Liu et al. (2010). Analysis of the biomass morphology by light microscopy showed distinct changes in the biomass structure and development of clear granular formations. There was also an observable reduction in the filaments protruding the surface of the granular structures which was consistent with previous findings by (de Kreuk et al., 2005).

Table 3: Process performance summary comparing AGS (Strategy A and B) and Full-scale SBR (Strategy C), showing the range (min-max) of nutrient removal performance (%) and biomass characteristics.

			PO_4 Removal (%)	NH_4 Removal (%)	Total Nitrogen Removal (%)	MLSS (g/L)	SVI_{30}	SVI_5/SVI_{30}
Strategy	A	(100% Anaerobic)	5.4–49.7	77.8–99.7	16.0–97.5	1.7–8.8	37.5–238.2	1.1–2.1
Strategy	B	(33% Anaerobic)	4.3–17.3	96.1–99.8	27.5–94.2	3.6–6.8	58.5–132.1	1.2–2.1
Strategy C (100% Aerobic)			n.d.	70.8–99.6	75.7–92.9	2.7–3.9	216.0–360.0	n.d.

The ammonia removal performance of all mature feeding strategies ranged between 70 and 99.7% with total nitrogen removal typically >75% (Table 3). Analysis showed decreased PO_4 removal in the 33% anaerobic feed AGS (strategy B), when compared to the 100% anaerobic contact operation (strategy A), with median PO_4 removals of 9.2 and 20.7%, respectively. The PO_4 removal efficiency in the anaerobic contact investigations was greatly reduced when compared to the removal efficiency observed in the full-scale CAS operation and other AGS trials conducted in low-saline environments (Bassin et. al., 2012). The nitrogen and PO_4 removal performance of AGS comparing both strategies A and B during start up is provided in the Supplementary Figure 1B and 1C.

Microbial Ecology

qPCR analyses compared key nitrifying and denitrifying microorganisms, with notable differences seen in the relative abundances of ammonium-oxidising bacteria (AOB), ammonium-oxidising archaea (AOA) and the NOB *Nitrobacter sp.* between the three feeding strategies (Figure 1A). The increased retention time (sludge age) of larger, denser granules can help explain the higher observed abundances of AOA within the AGS

biomass relative to full-scale aerobic CAS operation, with the oxygen and nutrient gradient most likely contributing to niche driven selective enrichment of slower-growing and less competitive archaeal ammonia-oxidisers over AOB (Short et al., 2013). Similarly, the development of the granular structures and increases in density and oxygen gradient may have driven the development of a nitrite-loop as seen by the increased abundance of *Nitrobacter sp.* (of 1-2 $\log_{10}$) and increased NOB/AOB ratio (by 20,000-50,000) within AGS. Similarly an increase in the proportion of NOBs was also observed by Winkler et al. (2012a) which occurred when denitrifiers supply NOBs with nitrite through the reduction on nitrate, thereby forming a nitrite-loop. In this study, we did not observe an accumulation of nitrite or nitrate in the effluent or reduced total nitrogen removal performance. The size separated biomass was also analysed using qPCR for the target functional genes (see Figure 1B). Notably, there was an increase in the abundance of AOA, *Nitrospirae* and denitrifying bacteria within larger granules (>1,400 µm), which was likely a result of the steeper oxygen gradient that exists across larger granules. Higher sludge age of the larger granules (>1,400; Figure 1B) may have further contributed to the increased abundance of AOAs in this size fraction, given the slower growth rate of AOA relative to AOB (Pronk et al., 2015).
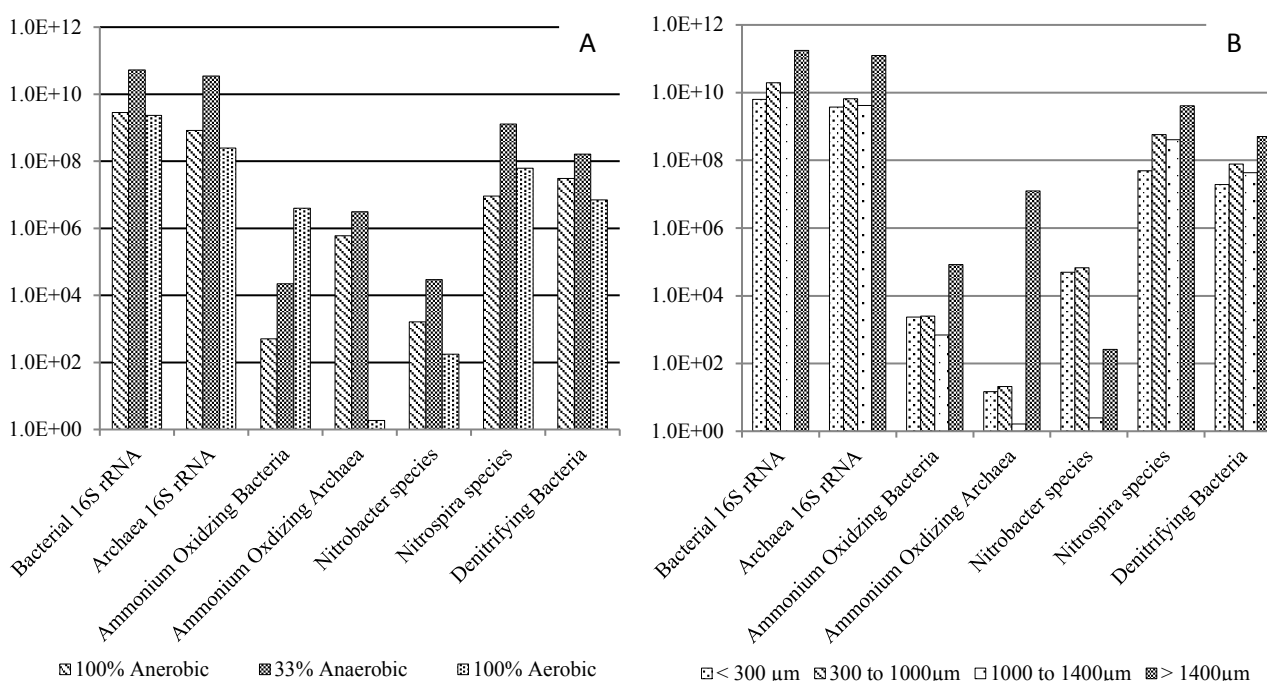

Figure 1: Changes in the functional microbial ecology of biomass samples as determined by qPCR, comparing **(A)** Strategy A (100% anaerobic feed) Strategy B (33% anaerobic feed) and Strategy C (100% aerobic feed); and **(B)** the impact of AGS granule size (<300 µm, 300–1,000 µm, 1000–1,400 µm and >1,400 µm) for Strategy B.

The increase in the abundance of *Nitrospirae* in the AGS samples seen within the qPCR was also confirmed through NGS sequencing, which showed an increase in abundance of phylum *Nitrospirae* organisms in samples taken from the two feeding strategies. Additionally, there was a clear increase in abundance of *Nitrospirae* in all granular morphologies when compared to the smaller floc like biomass (data not shown). This increase in abundance has previously been linked to the development of the oxygen gradient whereby a study by Guimarães et al. (2017) showed the localisation of *Nitrospirae* towards the core as the granule increased in volume, which corresponded with an increase in their abundance.

Analysis of the high throughput sequencing data showed increases in phylum *Proteobacteria* from 55.1% in the 100% aerobic strategy C, to 66.8 and 72.5 in strategy B (33% anaerobic) and strategy A (100% Anaerobic) systems. This phylum contains class *Betaproteobacteria* and *Gammaproteobacteria* microbes, with these two classes including the PAO *Candidatus phosphatis* and glycogen accumulating organisms, respectively

(Lemaire et al., 2008). Relevantly, these microbes are known to be associated with AGS development and granular formation through the production of EPS (Figure 2). Further analysis of this class showed increased abundance of *Betaproteobacteria* in strategy A (100% anaerobic), with the opposite in strategy B (33% anaerobic). Within this class, next generation sequencing (NGS) analysis showed the greatest enrichment of the PAO *Candidatus Accumulibacter phosphatis* occurred during strategy A (1.3% total community abundance), compared with 0.13 and 0.17% for strategy B (33% anaerobic feed) and strategy C (100% aerobic feed), respectively. This lower relative PAO abundance coincided with the reduced PO_4 removal performance observed within these systems. Furthermore, work by Wang et al. (2017) showed that the presence of increased abundances of *Proteobacteria* occurred within the high-saline granular sludge system while the abundance and metabolic activity of *Betaproteobacteria* decreased.
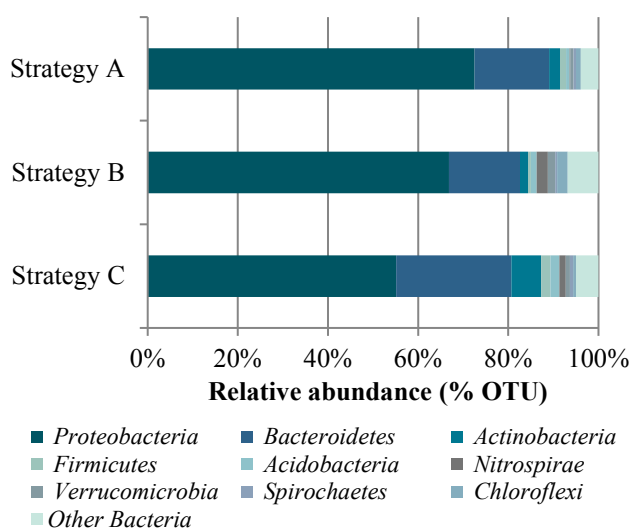
Figure 2: Comparison of the relative abundances of micro-organisms at the phylum level for each feeding strategy, which was determined using next generation sequencing of 16S rRNA.

In comparison to the CAS flocs, the class *Gammaproteobacteria* had increased by 18.4 and 20.8% during strategy A and B, respectively. This was largely attributed to a large enrichment in sulfide-oxidising bacteria (SOB) from the order *Chromatiales* and *Thiotrichaceae,* which collectively represented 23% of all operational taxonomic units (OTUs) compared to 6.6% within the CAS flocs. Size analysis of the separated granules found that the smallest granules had the highest representation of SOBs (Figure 3 A). It was possible that the uniquely high sulfate concentrations measured within the high-saline sewage (0.6-1.0 g/L) combined with the use of an anaerobic feed in the granular sludge pilot reactors, created conditions that favoured sulfate reduction, which provided a source of sulfide for the development of the SOBs. Following this, investigation of the sulfate-reducing bacteria (SRB) population showed no large increase in abundance of SRBs within the granular sludge biomass when compared to CAS (Figure 3 B). SRBs were however well represented at >1.8% of OTUs within all floc and AGS samples. The increase in abundance of the SOB population in AGS may indicate that these organisms played important roles in the development and stability of AGS in high-saline and high-sulfide wastewaters. In this system sulfide may be oxidised by SOB under aerobic and/or anoxic conditions (i.e. autotrophic denitrification) due to the existence of an oxygen gradient within the AGS granules. Furthermore Rubio-Rincón et al. (2017), recently showed that the SOB *Thiothrix* (a genus in the order *Thiotrichaceae*) could also play an important role in biological phosphate removal under high sulfide conditions. The role of SOB in AGS performance treating high-saline wastewater requires further investigation.
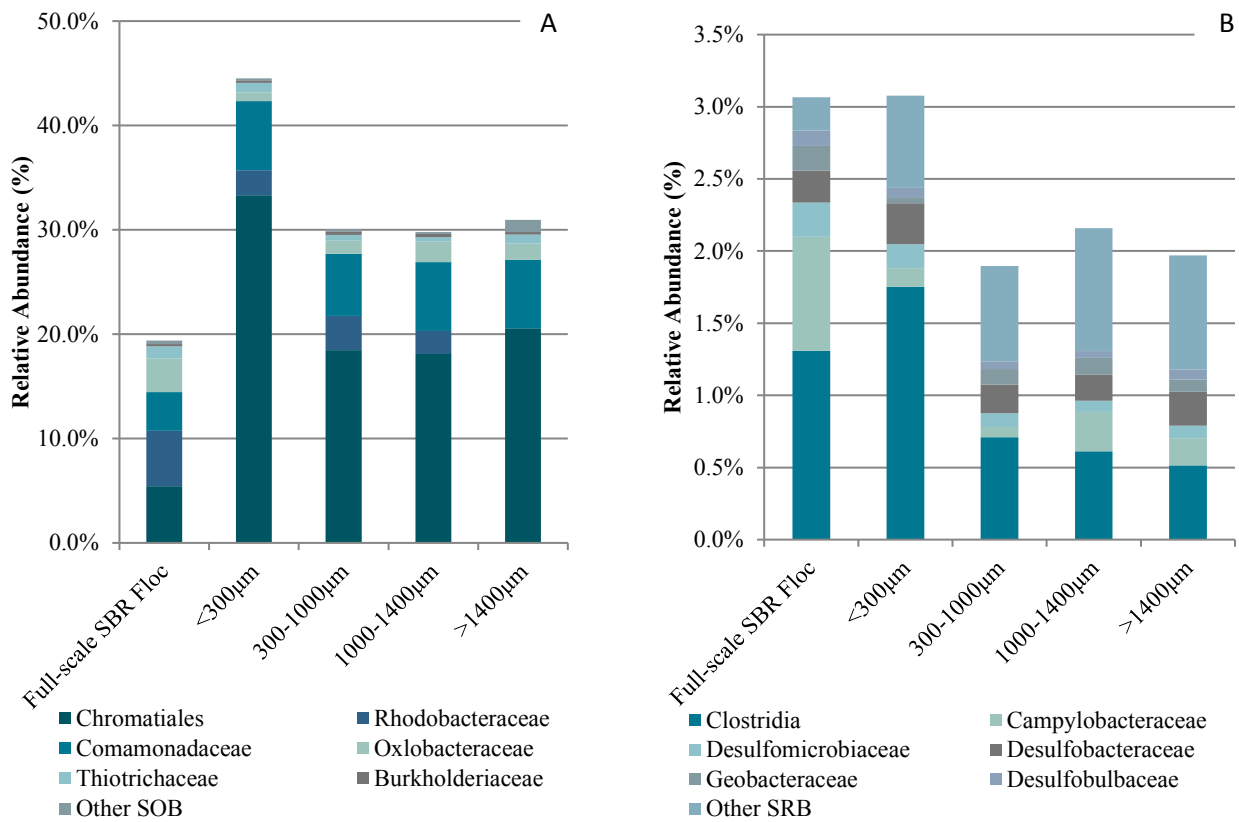
Figure 3: Changes in abundance of sulphide oxidising bacteria (A) and sulfate reducing bacteria (B) of size separated granules (<300 µm, 300–1000 µm, 1000–1,400 µm and >1,400 µm) that were sampled during Strategy B (33% anaerobic feed), with comparison to Strategy C (full-scale 100% aerobic feed).

Scanning Electron Microscopy (SEM) and X-Ray Florescence Microscopy (XFM)

SEM analysis comparing AGS and CAS samples identified very different external surface structures (Figure 4) and elemental composition. The AGS surface resembled microorganisms embedded in an exopolysaccharide-like crust. In contrast CAS was dominated by filamentous structures. EDS undertaken during SEM analysis indicated enrichment of sulfur on the surface of AGS, containing on average 2.9 At/wt% compared to 0.8 At/wt% detected on the CAS sample (Supplementary Figure 2).
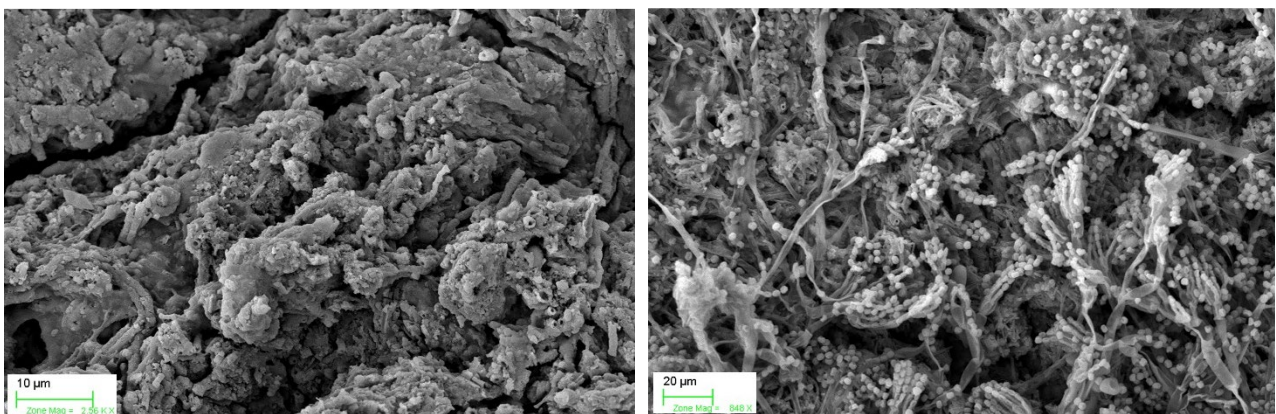
Figure 4: Scanning electron micrograms comparing the surface structure of (A) AGS granules taken from strategy B (33% anaerobic feed) and (B) biomass from strategy C (100% aerobic feed). Scale bar represents 10µm and 20µm respectively.

Elemental analysis of AGS using XFM (Figure 5) complemented the EDS analysis (Supplementary Information Figure 2) which showed hot spots of elemental sulfur (red) precipitation as well as localisation of copper (blue) and zinc (green), which appeared to have strong affinity to co-localisation (cyan) with no apparent interaction between sulfur and both copper (purple) and zinc (yellow). Further analysis showed the sulfur concentration was 2.99 wt% with the zinc and copper forming 0.038 and 0.046 wt%, respectively. The evidence of elemental sulfur deposition within the granules can potentially be explained by the increase in abundance of SOB within the AGS samples when compared to the CAS sample, given the oxidation of hydrogen sulfide by SOBs can result in the production of elemental sulfur or sulfate. While only preliminary at this stage and lacking a CAS comparator, XFM results together with EDS and NGS observations suggest a potential role for SOB in AGS under high-saline (high sulfate) wastewater applications that warrant further investigation.
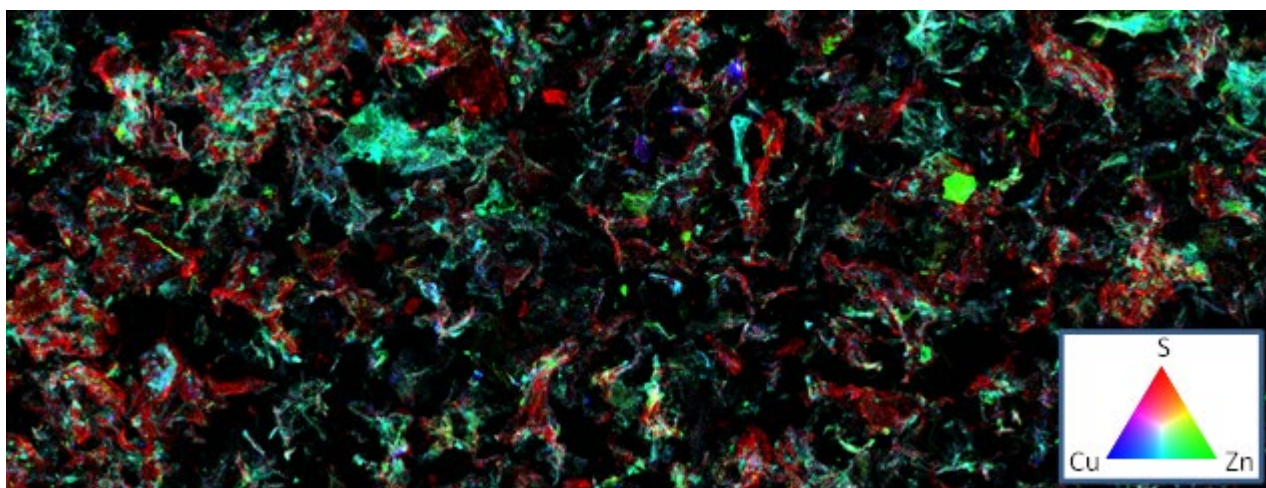

Figure 5: Tricolor map from the X-Ray Florescence Microscopy of AGS showing sulfur (red), zinc (green) and copper (blue).

CONCLUSIONS

This pilot study showed that the formation and stability of AGS treating high-saline wastewater may not be as critically dependent on long anaerobic feeding conditions as previously published research suggests. The start-up time, stability and performance of AGS in the split anaerobic/aerobic feed (Strategy B) was comparable to that with a dedicated anaerobic feed (strategy A). Analysis of the functional genes responsible for nitrification and denitrification showed changes in reactor functional microbial ecology between the two anaerobic feeding strategies, with no change in the nitrogen removal performance of the biomass. Reduced PO_4 removal performance was seen under partial anaerobic feed strategy B relative to the 100% anaerobic feed Strategy A, with high throughput sequencing suggesting that this was likely the result of reduced PAO abundance. The increase in abundance of SOBs in AGS indicates a potential role for these organisms in AGS development and stability, and warrants further investigation. This study has shown that AGS can be achieved and maintained with more challenging sewage characteristics such as those found in higher saline, high-sulfide municipal wastewater.

ACKNOWLEDGEMENTS

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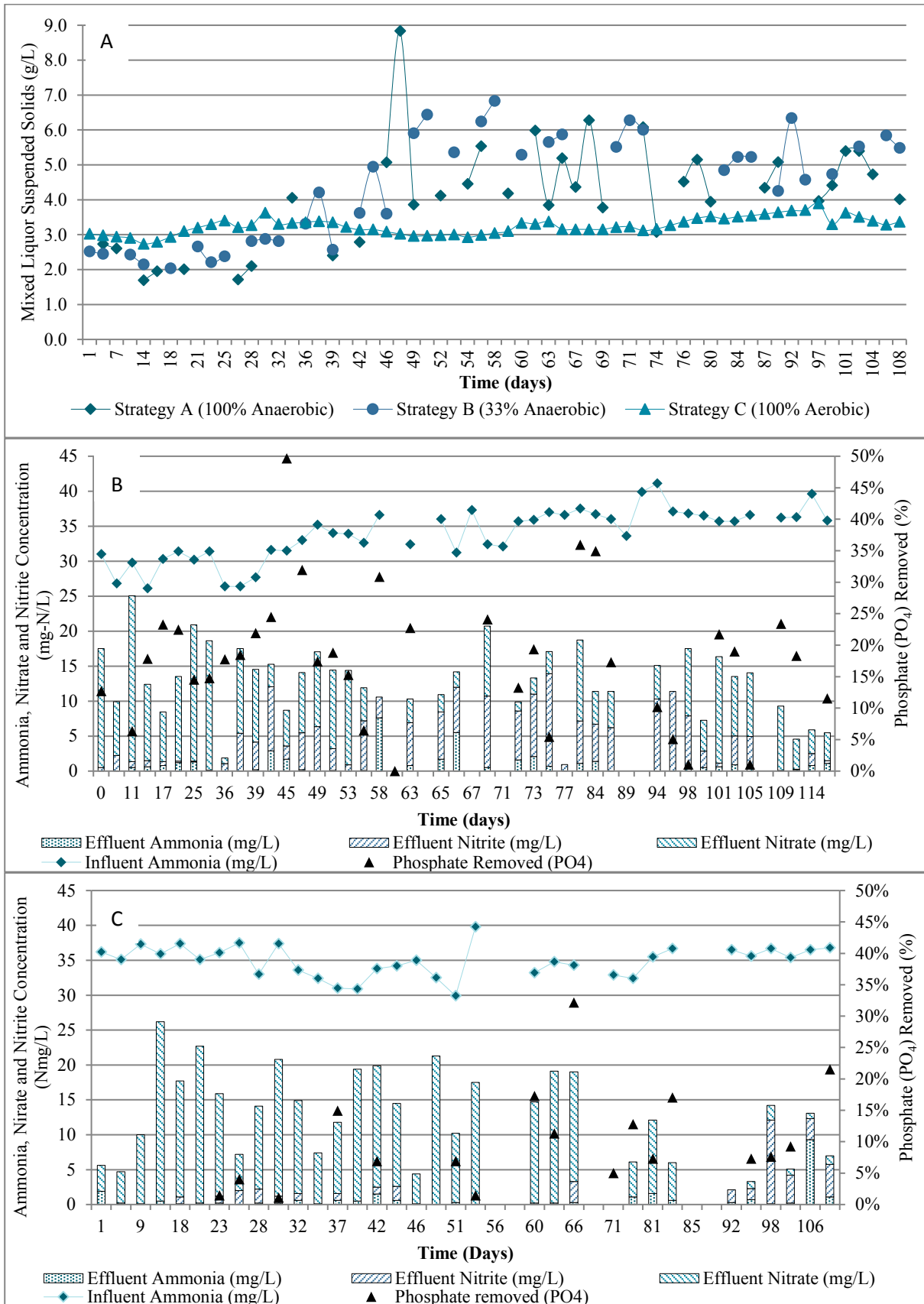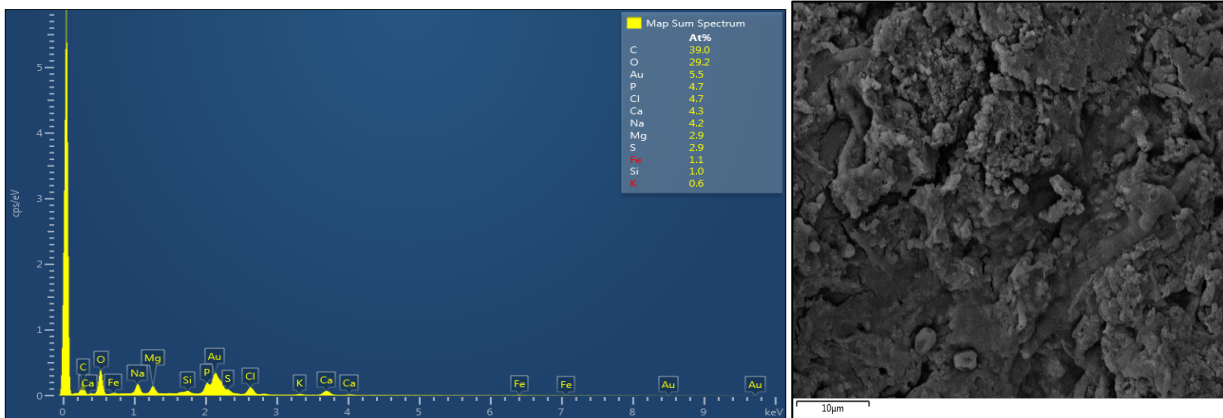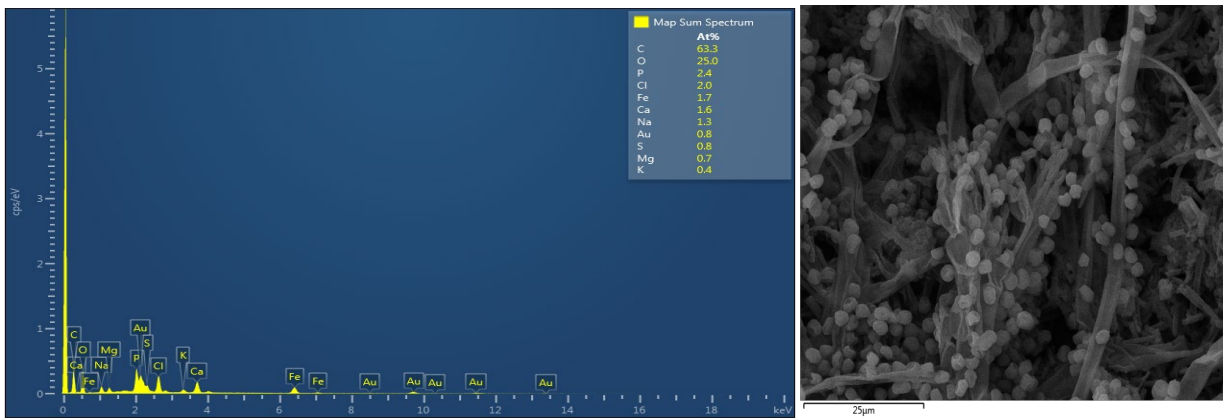
Supplementary Figure 1: Granular sludge influent ammonium-N concentrations, effluent concentrations of

ammonium-N, nitrite-N and nitrate-N and phosphate-P removal rates during start up comparing (A) Strategy A and (B) Strategy B. Figure 1C shows the development of AGS biomass in comparison the neighbouring full-scale SBR CAS biomass

A)

B)

Supplementary Figure 2. EDS spectrum comparing elemental composition of AGS biomass from (A) Strategy B (33% anaerobic feed) and (B) biomass from Strategy C (100% aerobic feed) and the corresponding SEM fields of view used for EDS analysis

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Title: Comparing the performance of aerobic granular sludge versus conventional activated sludge for microbial log removal and effluent quality: implications for water reuse

Authors: Benjamin J. Thwaites, Michael D. Short, Richard M. Stuetz, Petra J. Reeve, Juan-Pablo Alvarez Gaitan, Nirmala Dinesh, Ben van den Akker

Graphical Abstract:
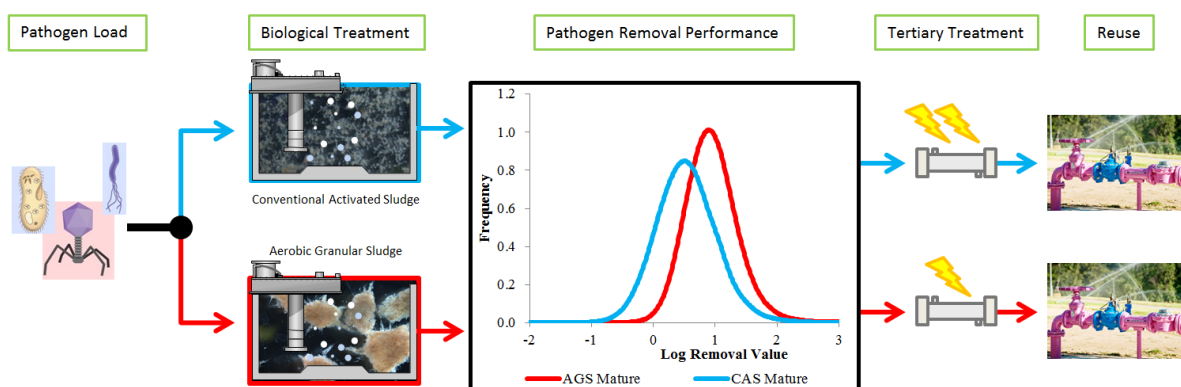
Abstract: The application of aerobic granular sludge (AGS) technology has increased in popularity, largely due to the smaller physical footprint, enhanced biological nutrient removal performance and ability to perform with a more stable operation when compared to conventional activated sludge (CAS) systems. To date, the ability of AGS to remove microbial pathogens such as; Escherichia coli, Giardia, and Cryptosporidium has not been reported. This study compared the log10 removal performance of commonly used pathogen surrogates (sulfite-reducing clostridia spores, f-RNA bacteriophage, E. coli and total coliforms) by AGS and CAS during the start-up phase, through to maturation. Results showed that AGS performed as well as CAS for the log10 removal performance of all microbial surrogates, except for spores which were removed more effectively by AGS due most likely to greater adherence of spores to the AGS biomass compared to CAS mixed liquor. Results suggest that AGS is capable of meeting or exceeding CAS-equivalent health-based targets for pathogen removal in the context of water recycling as well as not adversely affecting the secondary effluent water quality (suspended solids, turbidity and particle size) in terms of ultraviolet light transmissivity (254 nm). These findings confirmed for the first time that the adoption of AGS operation would not adversely impact downstream tertiary disinfection processes from altered water quality, nor would it require further pathogen treatment interventions in addition to what is already required for CAS systems.

Keywords: Aerobic granular sludge, biological nutrient removal, pathogenic indicator removal, wastewater recycling

1. Introduction

The use of aerobic granular sludge (AGS) in sequencing batch reactors (SBRs) has emerged as a viable alternative technology to traditional floc-based conventional activated sludge (CAS) for secondary level wastewater treatment. AGS conversion is achieved by modifying the operating parameters of SBRs to encourage CAS flocs to form dense, rapid-settling microbial granules. The conversion process has previously been shown to be heavily dependent on the application of an anaerobic feed combined with reductions in biomass settling time prior to decanting (Beun et al., 1999, de Kreuk, 2006, Pronk et al., 2015). Together, these changes allow for the selection of slow-growing microorganisms (polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs)) and washout of slow-settling microbial flocs, respectively (Bassin et al., 2012, Liu and Liu, 2006). This increased settling velocity of granular sludge allows for reduced cycling times (i.e. increased hydraulic capacity) resulting in reduced wastewater treatment

plant (WWTP) physical footprint (i.e. reduced infrastructure requirements) for new plants while increasing the hydraulic capacity of existing plants.

The role played by CAS processes for pathogen inactivation and removal from wastewater have been extensively studied (George et al., 2002, Koivunen et al., 2003, Lucena et al., 2004, Zhang and Farahbakhsh, 2007). These studies have shown that pathogens are removed through two main methods: predation; and/or biomass adsorption. Predation can be highly dependent on biomass characteristics (Raboni et al., 2016), with bacterial removal previously shown to occur primarily by protozoan grazing during the aerobic phase (Curds, 1973). This is in contrast to virus removal, which occurs primarily as a consequence of biomass adsorption (Bales et al., 1993, Gray, 1990). Viruses adhere to the surface of the biomass and subsequently subjected to microbially-mediated inactivation, or physically removed via sedimentation (Arraj et al., 2005, Ng et al., 1993b, Ng et al., 1993a). Configuration of CAS operations can also influence virus removal, with Tanji et al. (2002) showing increased adsorption of phage (bacterial viruses) when the biomass was exposed to anaerobic–aerobic treatments.

While the benefits of adopting AGS have been extensively researched and documented over the last ten years, there is no current knowledge of how changes in the biomass structure (from CAS to AGS) affects pathogen removal performance. This has particular importance in the context of water recycling, where secondary treatment processes such as CAS also function as a critical treatment 'barriers' for pathogen removal, enabling water authorities and regulators to satisfy part of the health-based targets for water reuse (Wen et al., 2009, NRMMC et al., 2006) thus reducing the extent of additional tertiary treatment requirements. In Australia, for example, wastewater treatment processes—including CAS—are commonly assigned $\log_{10}$ removal values (LRVs) based on verified bacterial, protozoan and viral pathogen removal performance when configured as part of water recycling schemes under the national Guidelines for Water Recycling (NRMMC et al., 2006). These guidelines outline the required scheme-wide LRVs necessary for a given reuse scheme and recycled water end-use. Under these guidelines, elements with variable configuration and operation such as CAS systems require validation to demonstrate that the process can provide effective and reliable pathogen reductions. Validation is often based on characterising the LRV performance of reference pathogenic microorganisms, or their appropriate surrogates (e.g. spores, bacteriophage, faecal coliforms) and is typically performed under anticipated normal operating conditions. Validation studies have shown that pathogen removal during CAS treatment can typically achieve bacterial, protozoan and virus removals of 1.0–2.2, 0.7–2.5 and 1.9–2.5 $\log_{10}$ respectively (Wen et al, 2009).

For water recycling schemes in particular, it is important to understand the performance of AGS in regards to pathogen removal, as any loss in LRV capacity may require additional tertiary treatment interventions in order to meet stringent health-based targets for intended end use or discharge for environmental flows. Alternatively, there would be additional requirements placed on irrigators to implement different methods of irrigation to control dispersion. Additionally, there is no information currently available on the relative pathogen removal performance of AGS during start-up conditions, information that is important for WWTP operators to understand due to the significant changes that occur with regards to both biomass morphology and surface characteristics, and subsequent effluent water quality. Furthermore, the downstream impacts of AGS operation on effluent water quality also need to be considered, as any adverse change in the treated water quality parameters such as total suspended solids (TSS), turbidity, colour, UV transmissivity (UVT) may impact on the performance and operational cost of UV and chlorine disinfection processes, which play an important role in delivering a robust multi-barrier approach to health risk management of recycled water. These impacts need to be investigated to ensure that any savings achieved by implementing AGS operation are not offset by the need for additional operational or capital expenditure due to loss in pathogen LRV performances or altered effluent water quality.

This study details the first investigation of pathogen LRV performance of AGS treatment (from start-up to mature operation), which was compared side-by-side with CAS operations at pilot scale. The variability in LRV performance was also characterised and linked to changes in effluent water quality, including UVT, SS and turbidity. LRV performance was based on the removal of four recognised pathogen surrogates in accordance with validation protocols of reuse schemes (Victorian Department of Health, 2013). This included sulfite-reducing clostridia (SRC) spores (conservative protozoan surrogate); f-RNA bacteriophage (human virus surrogate); *Escherichia coli (E. coli)*; and total coliforms (TC) (bacterial pathogen surrogates). These surrogates are widely used in validation exercises as they are typically more abundant in sewage than human pathogens and therefore allow high magnitude LRVs to be measured. The abundance and diversity of higher organisms in the two pilot reactors was also assessed, given the potential importance of predation on pathogen reduction in activated sludge systems.

2. Methodology

2.1 Pilot plant description

A pilot facility comprising two pilot reactors (Figure 1B) for CAS and AGS was established at a metropolitan WWTP in Adelaide, South Australia. The pilot plants used in this study have previously been described in detail by van den Akker et al. (2015). In short, pilot reactors had operating volumes of 61.5–63.5 litres and received untreated municipal sewage screened to ≈1 mm from the inlet of a neighbouring full-scale SBR plant. The typical feed sewage characteristics are shown in Table 1. SBR cycles of feed, aeration, settle and decant were controlled using a programmable logic controller. Both pilot plants were seeded with CAS harvested from a neighbouring full-scale SBR, to give an initial biomass concentration of 2 g/L when filled to operating volume.

Table 1: Typical influent sewage characteristics (in mg/L) of the high-saline municipal wastewater received at the full-scale SBR plant and pilot facility.

	TSS	NH_4^+-N	TN	PO_4^--P	COD
Mean ± 1 S.D.	150 ± 50	29.5 ± 2.8	30.6 ± 2.9	13.1 ± 1.71	414 ± 78.4
Range (min–max)	83–260	25.9–35.9	27.1–37.3	9.4–16.4	320–574

The CAS and AGS pilots operating conditions are given in Table 2. Briefly, the CAS pilot was operated with an aerobic feed and long settling time of 60 minutes to mimic the operation of the neighbouring full-scale SBR, whilst the AGS pilot was operated with an anaerobic feed and decreased settling time which had reached 12.5 minutes.

Table 2: Cycling conditions (in minutes) for both pilot plants.

	Anaerobic feed	Aerobic feed	Aeration	Settling	Decant	Total time
AGS Pilot	60	0	120	12.5–30	15–32.5	225
CAS Pilot	0	60	60	60	35	225

Loading and wastewater conditions for the CAS and AGS pilots are given in Table 3. The pilots were filled aerobically (CAS) or anaerobically (AGS) dependant on desired operating outcome with a volumetric exchange ratio of 25%. The dissolved oxygen (DO) concentration in the CAS and AGS pilots was maintained between 1.0–2.5 mg/L and 0.5–2.5 mg/L respectively for the duration of the trials (113 days). Settling time was reduced in the AGS pilot from 30 to 12.5 minutes over 74 days (immature phase days 0–73) with the pilot running 39 days (mature phase days 74–113) continuously at this settling time.

Table 3: Pilot plant performance data during the 113 day study (data in parentheses shows minimum and maximum performance range for each parameter)

	Dissolved oxygen (mg/L)	Organic load (COD kg/m³/d)	Nitrogen load (TN kg/m³/d)	MLSS (g/L)	Temperature (°C)
AGS Pilot	0.5–2.5	0.50	0.047 (0.042–0.057)	2.95 (1.77–4.67)	23.6 (18.8–27.2)
CAS Pilot	1.0–2.5	0.50	0.049 (0.043–0.059)	2.37 (1.66–2.75)	23.4 (15.2–25.9)

2.2 Monitoring and analysis

Wastewater treatment performance was monitored during the trial through the measurement of influent and effluent NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^--P and COD using commercial HACH® test kits (10031, 10019, 10020, 8048 and 8000 respectively). Total dissolved solids were analysed using HACH sensION 7 (USA). Mixed liquor was analysed for suspended solids (MLSS), effluent TSS and settleability using a 30-minute sludge volume

index (SVI_{30}) following standard methods (APHA, 1998). Changes in mixed liquor morphology were observed by light microscopy (Nikon, SMZ1000). Filamentous bacteria and higher organism identification and enumeration was conducted on CAS and mature AGS biomass samples (n = 3) at the Australian Water Quality Centre (Adelaide, Australia) according to the methods of Eikelboom (2000) and Jenkins (2003).

UVT analysis was conducted using a GENESYS 6™ UV-Vis spectrophotometer (Thermo Electron Corporation, Madison, Wisconsin, USA), with 5 mL samples placed into the quartz cuvette and absorbance analysed at 254 nm. Turbidity was measured using 2100N Turbidimeter (HACH). 10 mL samples were placed into the glass cuvette, inverted several times to mix, then analysed and reported as nephelometric turbidity units (NTU).

2.3 Sampling program

Grab samples of screened influent sewage, mixed liquor and treated secondary effluent were taken periodically during the trial phases. Influent sewage was sampled from an access point located immediately prior to the pilot reactors, mixed liquor samples were collected during aeration and effluent samples were collected from two locations: location A decant point (Figure 1, location A); and location B in to replicate a surface decant weir sample location used by full-scale SBRs (Figure 1, location B). Samples were collected between the hours of 0700 and 1100 and stored at <4°C prior to same-day microbial analyses.
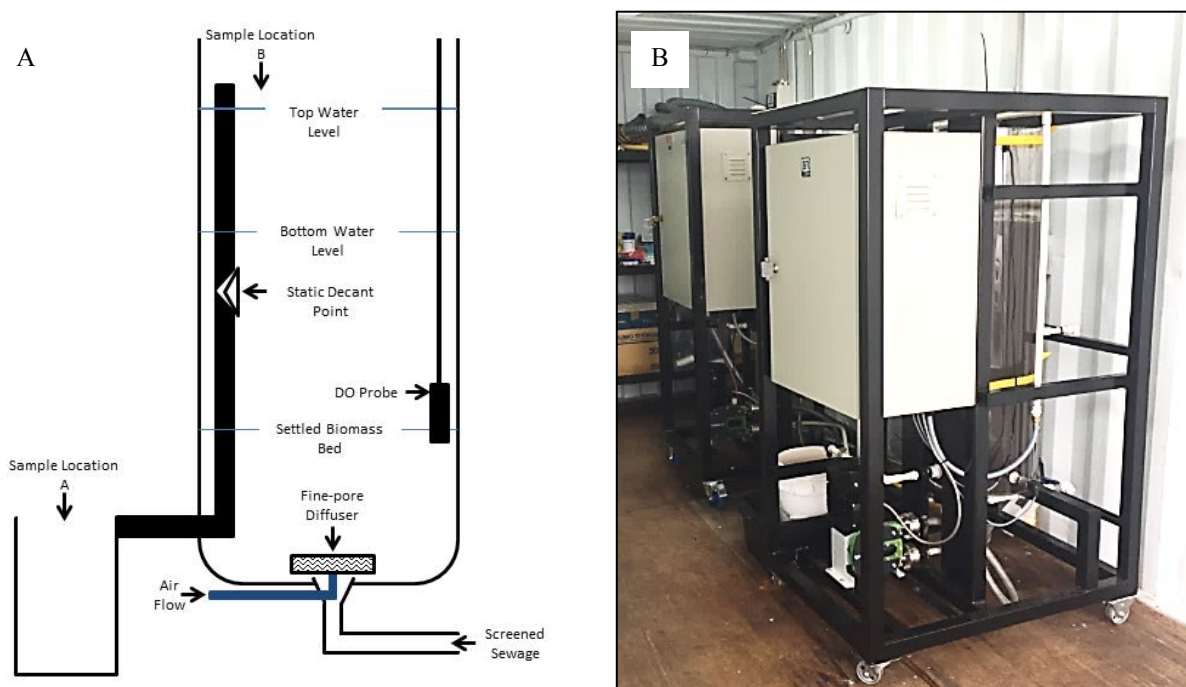

Figure 1: Schematic of the SBR pilot plants (A) operated for both AGS and CAS, showing the two effluent sampling locations (A and B) and (B) side-by-side pilots onsite

2.4 Microbial indicator analyses

Indicator bacteria (*E. coli* and TC) and the more conservative sulfite-reducing clostridia (SRC) spores were assayed as outlined below after serial dilution of samples in sterilised UltraPure (Barnstead) water. For *E. coli* and TC, 100 mL of diluted sample was filtered through 0.45 µm gridded filter membranes (47 mm, Millipore, S-Pak), with the membrane placed onto the surface of a dried plate of selective agar (Brilliance, Oxoid CM1046) and inverted and incubated aerobically at 37°C for 24–36 h. *E. coli* was enumerated by counting blue colonies, while TC were enumerated by counting both purple and blue colonies. *E. coli* colonies were confirmed by negative reactions to Oxidase Test Strips (Oxoid, MB0266). SRC spore abundance was assessed on agar plates using Perfringens Agar Base (Oxoid CM0587) plus Perfringens Selective Supplement (SR0088) for *Clostridium perfringens*. Samples were filtered through 0.45 µm gridded filter membranes (47 mm, Milipore, S-Pak) and incubating inverted anaerobically at 35 ± 1°C for 24–36 h. Prior to dilution a 50 mL

aliquot of each sample was heated to 70°C for 20 minutes to inactivate vegetative cells (Adcock and Saint, 2001). *E. coli,* TC and SRC abundance was expressed as colony-forming units (CFU) per 100 mL.

f-RNA bacteriophage was quantified using the double agar layer technique as outlined in Noble et al. (2004). Briefly, E. coli F-amp host (ATCC #700891) was cultured in TSB until growth phase was established (4-6 hours), this was confirmed using absorbance spectrophotometry and pre-determined optimal range. 100 µL of *E. coli* culture was added to molten tryptone soya agar (TSA) overlay containing 3mL of sample. Overlay samples were inverted to mix and then spread evenly over dried TSA plates impregnated with 10 mg/L Ampicillin (Sigma-Aldrich). Agar overlays were allowed to cool and set before being inverted and incubated aerobically for 24 h at 37 ± 1°C. Phage numbers were determined by counting plaques (cleared zones) >2 mm and final abundance were expressed as plaque-forming units (PFU) per 100 mL. All microbial indicator abundance data was $\log_{10}$ transformed for presentation and further analysis.

2.5 Filamentous and higher organism analysis

Enumeration of filamentous bacteria was performed at the Australian Water Quality Centre (Adelaide, Australia) using the in-house testing method TM-054, which was based on conventional microscopy of the morphological features using the manuals by Eikelboom (2000) and Jenkins (2003). The relative abundance of 21 types were assessed qualitatively using a scoring to characterise filament abundance ranging from 0 (none) to 6 (excessive). Every sample was analysed by the same specialist to maintain consistency.

Higher organisms (namely protozoa and macroinvertebrates) were enumerated. Spirochaete and Zooglea sp. were analysed in parallel to filaments. Enumeration was based on conventional phase contrast microscopy of the morphological features using the methods described by Lindrea et al. (1999). The relative abundance of protozoan (free swimming ciliates, stalked ciliates, flagellates, suctoria, naked amoeba, testate amoeba and swimming amoeba), macroinvertebrates (rotifers and nematodes) and other organisms (spirochaetes and zooglea sp.) were scored semi-quantitatively ranging from absent (-); 1-2 observed (-/+); 5-10 observed (+); 10-100 observed (++); and >100 observed (+++).

2.6 Statistical analyses

Changes in nutrient removal performance were examined by nonparametric ANOVA (Brown-Forsyth ANOVA and Bartlett's t-test) analysis (immature AGS, Mature AGS and Mature CAS comparisons), unpaired t-tests (Mann-Whitney) were utilised to investigate the change in microbial indicator removals and effluent quality (suspended solids, UVT and Turbidity). Statistical significance was accepted at the $p<0.05$ level, with all analyses conducted using PRISM 6 (Version 6.07, GraphPad software, California, USA). Similarity of the filamentous bacteria and higher organism ecology in AGS and CAS were assessed using Bray-Curtis similarity analysis using Primer 6 (Primer-E, Pylmouth, UK) using average observed abundance values.

2.7 Probability density function (PDF) fitting

Microbial indicator data variability within the sampled influent, mixed liquor and decanted effluent was characterised by fitting exceedance probability plots. This form of analysis was utilised as it describes the data variability as used to inform quantitative microbial risk assessments. Indicator abundance was fitted with a normal probability distribution using @Risk software (Palisade Corporation, version 7.5) in order to perform a Monte Carlo simulation to determine the variation in indicator removal performance between immature AGS, mature AGS and the matured CAS pilots.

2.8 Biomass homogenisation

Mature AGS and CAS biomass samples were homogenised to detach microbial indicators in order to assess and compare the relative partitioning of microbial indicators between the liquid (wastewater) and solid (biomass) phases. Biomass samples (n = 4) were homogenised in the presence of a 1× Zwittergent 3-12 (Merc-Millipore) at 8000 rpm (WiseTis Homogeniser, Thermoline Scientific) for a total of 4 minutes, with homogenisation being completed in one minute intervals (Caron et al., 2007). Indicators were measured before and after biomass homogenisation using methods outlined in Section 2.4 for un-homogenised samples.

2.9 Particle sizing

Particle size distribution (0.37–460.27 µm) was analysed using 100 mL CAS and AGS pilot reactor effluent samples collected from sampling location B (Figure 1) using a LISST-Portable instrument (Sequoia Scientific, USA). Full-scale secondary effluent was also collected and analysed to allow for direct comparison with the pilot scale data. Effluent samples were analysed in undiluted form, while non-homogenised and homogenised mixed liquor samples were diluted in sterile water to within acceptable analytical limits for the instrument. All samples were analysed in triplicate and the instrument chamber was rinsed with sterile ultrapure water (Barnstead) between samples. Particle size distribution was analysed using LISST Portable XR software, with final particle abundance given as number particles/L.

3. Results and Discussion

3.1 Pilot facility start-up and maturation

Figure 2 compares the SVI_{30} for both the AGS and CAS pilots over the study period (113 days). Analysis of the sludge settleability showed significant improvement in settling velocity for the AGS pilot over the initial 58 days of operation. During this time, AGS was achieved using the biomass washout technique whereby the settling time was reduced from 30 minutes down to 12.5 minutes. The formation of granules coincided with an increase in the biomass concentration (3.09 g/L to 4.67 g/L) with the AGS system and was considered to be mature from day 74 where it maintained an average SVI_{30} of 92.3.

In contrast to the AGS results, the SVI_{30} of the CAS system was consistently higher during the entire trial and was statistically similar to that of the full-scale SBR ($t_{(44)}$=0.291; p=0.134) with average SVI_{30} of 282.9 and 286.7 mL/g MLSS respectively. For comparison, Figure 2 also shows the full-scale SBR SVI_{30} over the same period, with results showing that both pilot and full-scale CAS plants were operating with similar sludge settling characteristics.
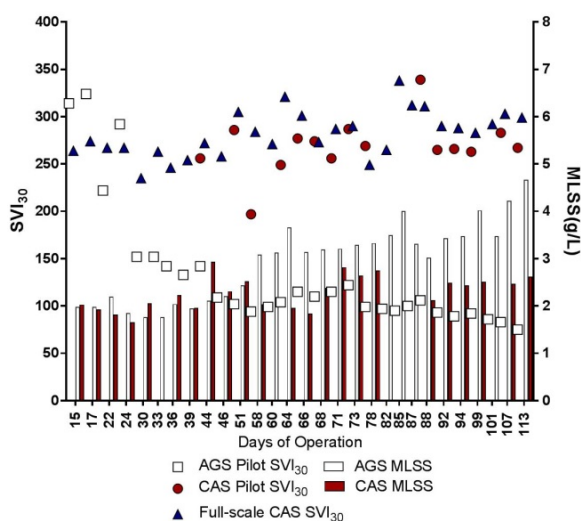

Figure 2: Biomass settleability (SVI_{30}; symbols) and mixed liquor suspended solids (MLSS; bars) for AGS, CAS and full-scale CAS SBR.

3.2 Nutrient removal performance and mixed liquor morphology

The nutrient removal performance of the pilots during mature (CAS) and immature (0–74 days) plus mature (75–113 days) (AGS) operation are summarised in Figure 3. For the AGS pilot, and despite distinct changes in biomass morphology from immature flocs to mature granules (Figure 4), nitrification performance was consistently high, as evidenced by the >95% ammonium removal performance throughout the trial. These

NH$_4^+$-N removals are consistent with previously published studies (de Kreuk et al., 2005, Bassin et al., 2012) despite the change in morphology. Statistically, NH$_4^+$-N removal performance was similar between the AGS and CAS pilots, irrespective of operational maturity ($F_{(2,34)}$=1.03; p=0.368).

Total nitrogen removal efficiency, although statistically similar between AGS and CAS pilots ($t_{(35)}$=0.350; p=0.599), was quite variable and was likely attributed to the over-aeration in some cycles due to limitations with the pilot facility aeration control system, leading to impaired denitrification and oxidised nitrogen (NO$_3^-$-N) accumulation similar to those observed in previous studies (Thwaites et al., 2017, van den Akker et al., 2015) (Supplementary Figure 1).

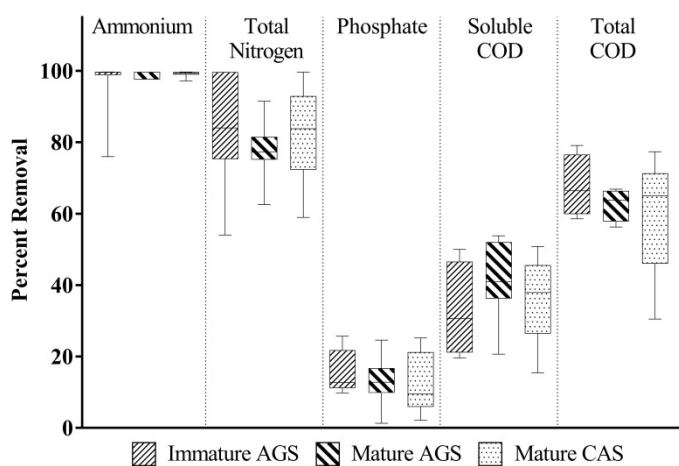Figure 3: Treatment performance data (100–(C$_{effluent}$/C$_{influent}$)×100) for immature (0–73 days) and mature (74–113 days) AGS and CAS pilots over the trial period (chart represented mean ± 1 S.D).

Soluble phosphate-phosphorus (PO$_4^-$-P) removal was similar between both AGS and CAS pilots ($t_{(28)}$=0.880; p=0.386) regardless of biomass maturity. Net phosphate removals in the AGS reactor were markedly reduced when compared to the expected >90% removals seen in other studies (Bassin et al., 2012, de Kreuk et al., 2005, Pronk et al., 2014) and Winkler et al. (2011) who showed P-removal efficiency of 71 ± 5%. This may be indicative of the AGS being dominated by GAOs over PAOs (Winkler et al., 2011). Soluble COD removals were statistically similar between the AGS and CAS pilots ($t_{(24)}$=0.485; p=0.632) at around 35–40%, with the total COD removals also similar on average ($t_{(9)}$=0.676; p=0.52) at between 60–63%.

Morphological analysis of mixed liquor from pilot reactors via light microscopy revealed a distinct change in appearance with the conversion of CAS to AGS operation (Figure 4). Figure 4 shows the changes in mixed liquor morphology when examined using light microscopy with the distinct granular structure being observed by day 74 and maintained until the end of the trial. Figure 4A shows the mixed liquor that was used to seed the pilot operations, Figure 4B and C shows the biomass morphology at day 113.
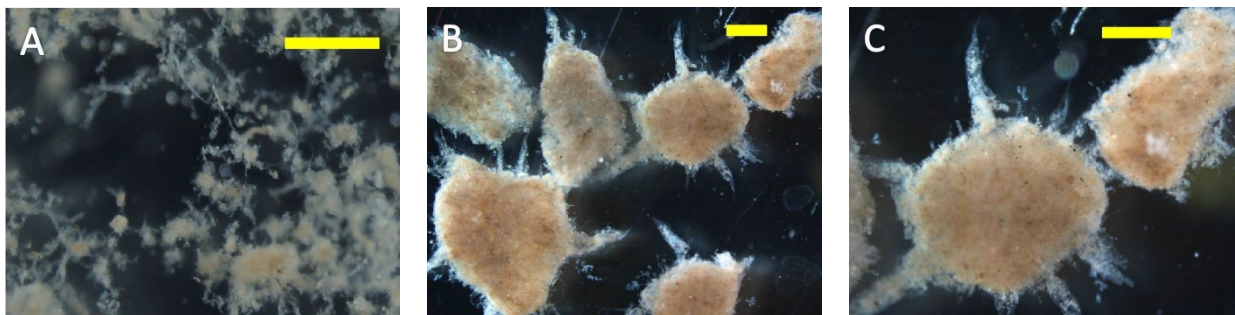

Figure 4: Mixed liquor suspended solids at day 0 (A) and at day 113 (B + C) from the AGS pilot. Scale bar represents 100μm

Morphological analysis of mixed liquor from pilot reactors via light microscopy revealed a distinct change in appearance with the conversion of CAS to AGS operation (Figure 4). Figure 4 shows the changes in mixed

liquor morphology when examined using light microscopy with the distinct granular structure being observed by day 74 and maintained until the end of the trial. Figure 4A shows the mixed liquor that was used to seed the pilot operations, Figure 4B and C shows the biomass morphology at day 113.

3.3 Sampling location effects

It is important to note that both of the pilot reactors were built with a static decant outflow point that was situated above the settled sludge bed (Figure 1A). It was recognised that the effluent quality taken from this decant location may not be representative of treated secondary effluent that is collected from a surface decant weir which are common with most SBR installations. For example, there is potential for elevated suspended solids being discharged due to the close proximity of the decant point to the settled sludge bed, which can also result in increased abundances of viable pathogens and indicators. Parallel grab samples were therefore collected from the top of the reactors (Figure 1, Sample location B) to simulate off-take from a surface decant weir. Statistical analyses was then conducted comparing effluent concentrations of microbial indicators enumerated from the two decant locations and this showed that there were no significant differences between the two sample points for both CAS and AGS for all of the surveyed indicator organisms (Supplementary Table 1). Since both sample points produced statistically similar indicator organism abundances, data points from both sample locations were pooled for analysis and presentation hereafter.

3.4 Abundance of microbial surrogates

Abundances of microbial indicators were assessed within the influent sewage, biomass (MLSS) and treated secondary effluent of both CAS and AGS pilot plants using frequency distribution probability plots (Figure 5). Having data in this format provides an appreciation for inherent variability of treatment performance. Average concentrations of *E. coli*, TC and SRC spores in the influent sewage were 5.31×10^7, 5.15×10^8 and 3.56×10^5 CFU/100 mL respectively; while influent f-RNA phage concentration was on average 9.56×10^4 PFU/100 mL (Figure 5). This analysis also showed that there was an average increase of approximately 1-log in abundance of SRC spores present in the AGS (1.38×10^6) and CAS (1.58×10^6) biomass samples when compared to the influent sewage. This accumulation was in contradiction to the other indicators analysed which showed approximately 1-log decrease in the biomass samples, indicating the potential for SRC spores to adsorb and accumulate within the biomass. Analysis of the effluent samples taken showed concentrations of f-RNA concentrations were from the AGS pilot showed the average abundance of 2.72×10^2 (AGS) and 4.17×10^2 (CAS) PFU/100mL for f-RNA, 3.50×10^4 (AGS) and 7.54×10^4 (CAS) CFU/100 mL for SRC, 3.11×10^4 (AGS) and 8.43×10^4 (CAS) CFU/100mL for *E. coli,* 2.38×10^5 (AGS) and 6.63×10^5 (CAS) CFU/100 mL for TC
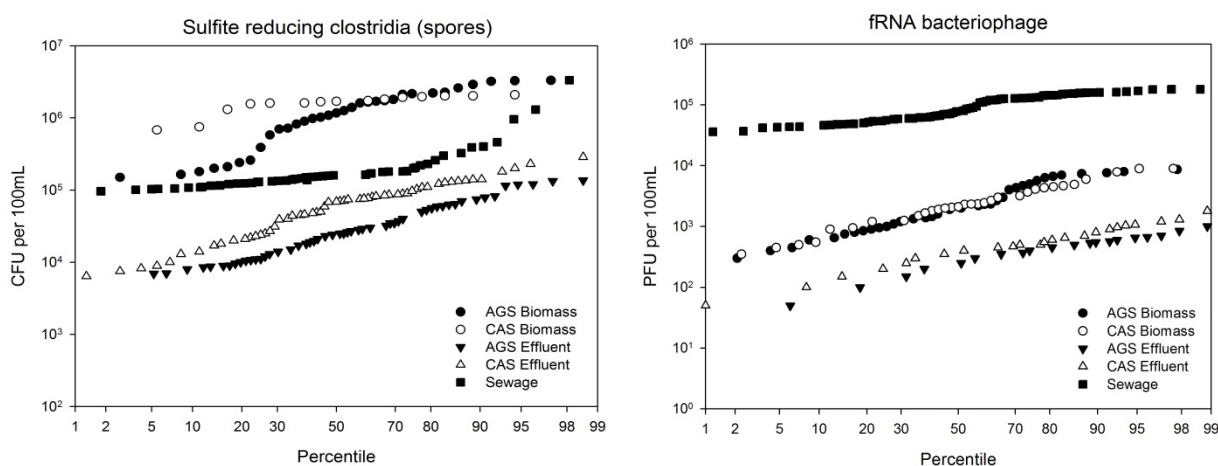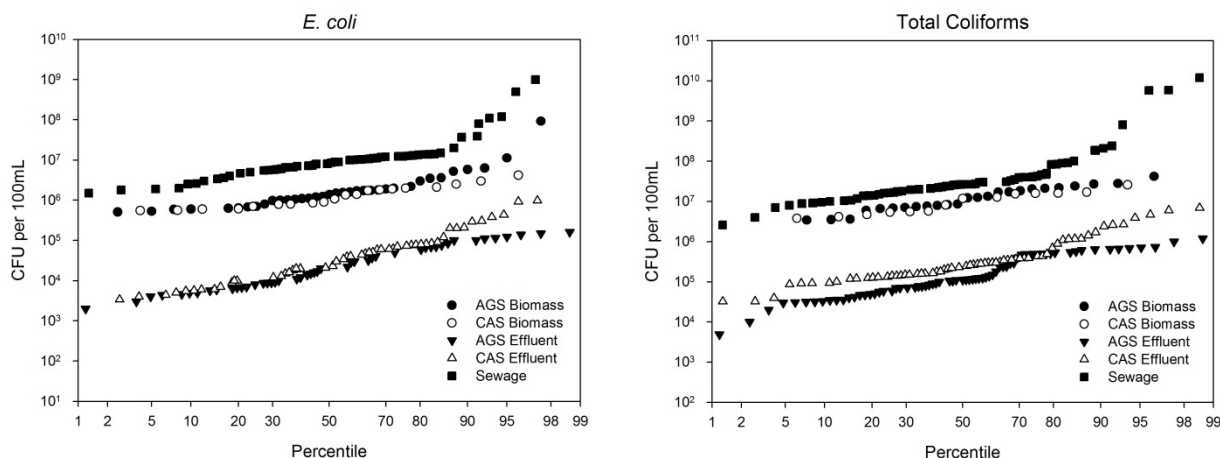
Figure 5: Frequency distribution plots showing the abundances of microbial surrogates within the sewage, mixed liquor and effluent of CAS and AGS pilot plants.

3.5 Log$_{10}$ removal performance of microbial indicators

Log$_{10}$ removal value (LRV) indicator performance PDF plots for both the CAS and AGS pilot (both immature and mature AGS) are presented in Figure 6. Monte Carlo simulation PDF fitting of all indicators enumerated from the raw sewage and decanted effluent was successfully achieved by applying a normal distribution. Median LRVs for *E.coli* and TC were only slightly higher for AGS (2.6- and 2.4-log$_{10}$ respectively) when compared to CAS (2.4- and 2.0-log$_{10}$ respectively). These values also compared well to the removal performance for CAS and secondary processes for f-RNA, *E. coli* and TC obtained from previously reported validation trials (Wen et al., 2009, NRMMC et al., 2006). Median LRV for f-RNA phage was the same for mature AGS and CAS at 2.4-log$_{10}$ (Figure 6B). Interestingly, the median f-RNA phage LRV performance of the immature AGS (0–73 d operation) was 0.37 log$_{10}$ greater than the mature AGS and CAS systems. To better understand the effects of AGS biomass morphology on pathogen removal, LRV performance was plotted over time from start-up to day 113 (Supplementary Figure S2). For SRC spores, LRV performance significantly improved around day 45–55 (Supplementary Figure 2B) which coincided with the time of granule maturation. An explanation for this improved LRV performance is unclear; however, it is possible that changes in biomass surface properties that occur during AGS start-up and maturation, such as increased EPS production (Liu et al., 2004, McSwain et al., 2005, Wingender et al., 1999), may have enhanced the potential for pathogen biomass attachment. For example, adsorption is considered a major removal mechanism of viruses in CAS systems (Wolfaardt et al., 1999), with previous work examining virus removal by CAS showing that virus interactions change with altered biomass morphology (Schijven et al., 2003).
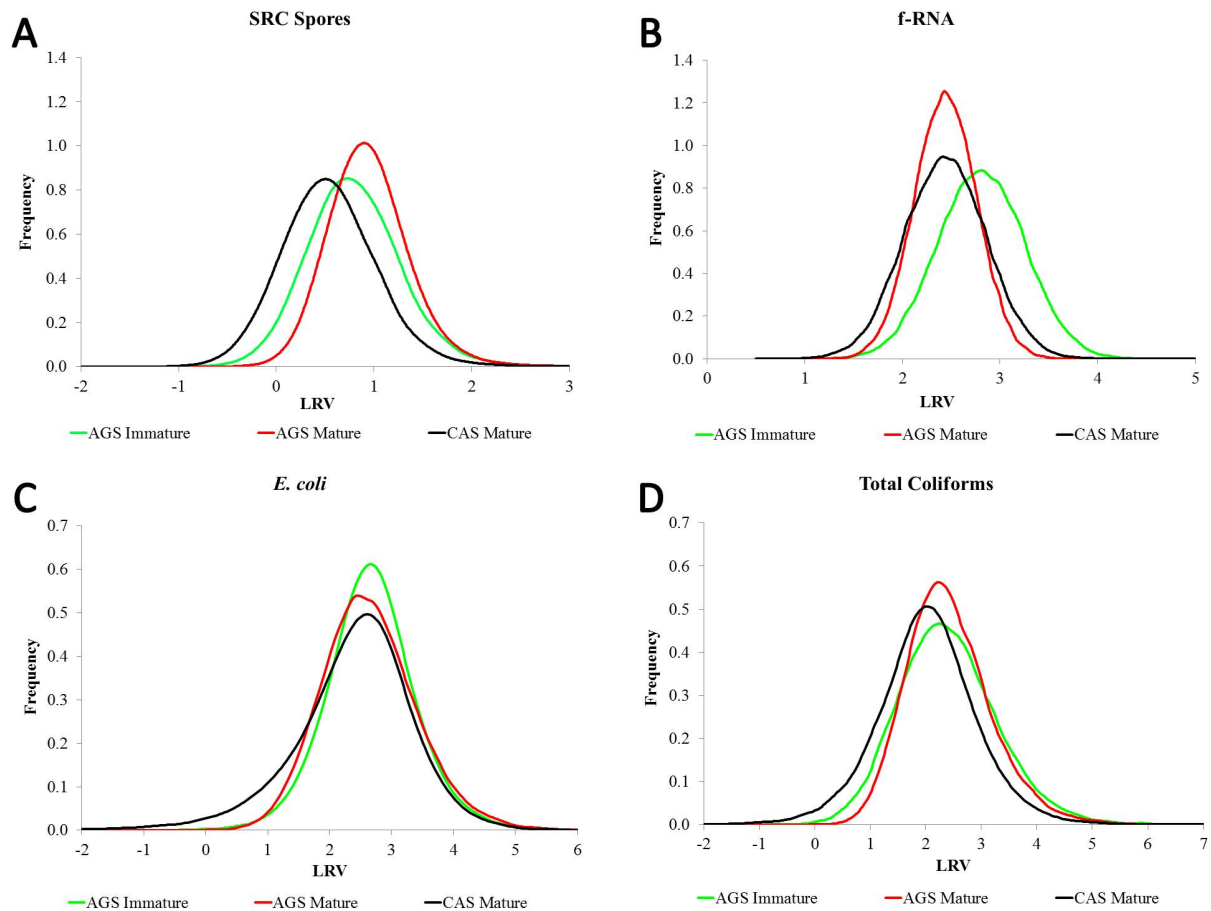
Figure 6: PDFs fitted using Monte Carlo simulations showing the log$_{10}$ removal values (LRV) for immature AGS (green); mature AGS (red) and CAS (black) for all microbial surrogates.

The LRV of SRC spores (Figure 6A) was lower than that of the surveyed vegetative indicator (*E. coli* and TC) bacteria and phage in both CAS and AGS and therefore can be viewed as a conservative estimation for the worst-case removal performance benchmark for risk assessment purposes. The median LRV of SRC spores was however greater in the mature AGS (0.91-log$_{10}$) when compared to CAS (0.46-log$_{10}$) and immature AGS (0.78-log$_{10}$) (Supplementary Table 2). Given anaerobic spores are known for their ability to strongly adhere to biomass and resist predation, the nature of this improved LRV performance of AGS over CAS was further investigated in Sections 3.6 and 3.7.

While mean LRVs for *E. coli* (2.64 and 2.38) and TC (2.42 and 2.02) were similar between CAS and AGS respectively, probability plots showed that LRV variability was moderately higher in the CAS system (Figure 6C and D). When comparing 5th percentile LRV, the mature AGS outperformed CAS with regards to *E. coli* (1.45 and 0.64), TC (1.29 and 0.56) and SRC spores (0.31 and -0.27) by a LRV difference of 0.6–0.8-log$_{10}$ (Supplementary Table 2).

3.6 Indicator organism adsorption potential of biomass

Initially, the biomass samples from CAS and AGS were homogenised in order to compare the role of adherence/adsorption of microbial indicators to biomass. Secondly, comparison of higher organism abundances in CAS and AGS was also done to see if the potential for predation differed between the two systems. Samples of AGS and CAS were homogenised to detach phage, spores and bacteria to investigate and compare the potential role of biomass adsorption and accumulation in the removal of indicator organisms. The abundances of indicator organisms were calculated and compared in both homogenised and non-homogenised samples (Figure 7). Homogenisation of biomass samples from both mature AGS and CAS

showed increased abundances (release) of all microbial indicators following the homogenisation treatment (Figure 7). Following homogenisation of AGS mixed liquor, organism abundances increased by 1.05-$\log_{10}$ for SRC spores ($t_{(35)}$ = 5.17, p<0.0001), 0.92-log for TC ($t_{(16)}$ = 4.95, p<0.0001), 0.52-$\log_{10}$ for *E. coli* ($t_{(14)}$ = 1.84, p=0.09) and 0.08-$\log_{10}$ for f-RNA bacteriophage ($t_{(28)}$ = 1.81, p=0.08). Homogenisation of the CAS mixed liquor gave 0.35–0.36-$\log_{10}$ increases for SRC spores ($t_{(23)}$ = 4.79, p<0.0001), *E. coli* ($t_{(14)}$ = 3.17, p=0.007) and f-RNA phage ($t_{(28)}$ = 9.48, p<0.0001) and a 0.54-$\log_{10}$ increase for TC ($t_{(15)}$ = 3.03, p=0.008). Alternatively, the homogenisation process may not have been significantly aggressive enough in order to successfully detach the phage.
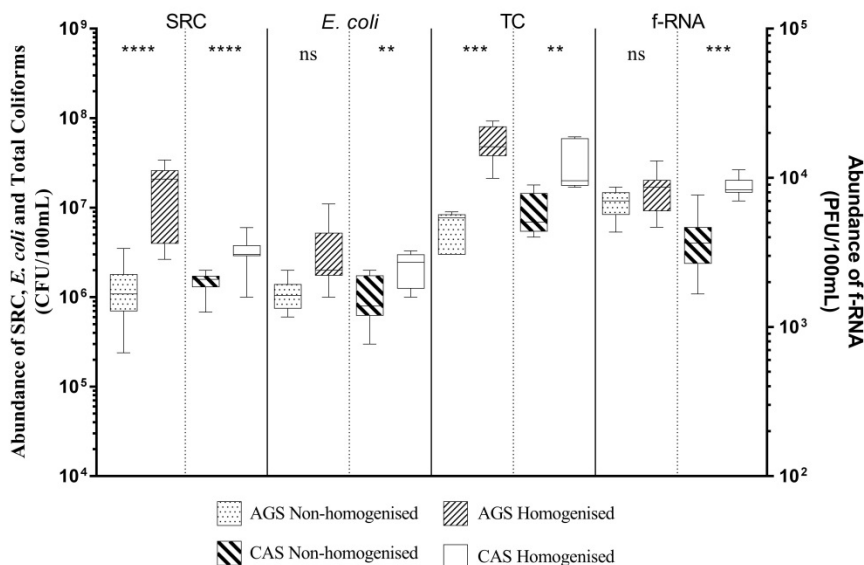

*Figure 7: Results from homogenisation of mixed liquor in the presence of a 1x Zwittergent showing significance *>0.05, **>0.01, ***>0.001 and ****>0.0001*

The higher release of SRC spores, *E. coli* and total coliforms measured following homogenisation of the AGS biomass suggests that the AGS exhibited an increased potential of adherence of microbial surrogates when compared to the CAS mixed liquor. This effect was greatest with SRC spores, with AGS displaying higher potential for the entrapment of spores, which may explain the superior LRV performance by AGS (Supplementary Table 2). These results indicate that higher partitioning of indicators into AGS biomass may occur, which may be a potential advantage of AGS. While the homogenisation technique used here is an accepted method to dislodge microorganisms bound to particulate material (Caron et al., 2007, Li et al., 2009), it is however unable to distinguish between microbial indicator organisms that are attached to the surface of AGS versus those that might be entrapped internally as the AGS develops. For the SRC spores, the possibility of internal entrapment is of interest and worthy of further investigation, given the ability of spores to withstand long periods of dormancy. While increased partitioning into the biomass is an advantage for secondary effluent disinfection, there is potential for the increased load to be impacting downstream sludge treatments including thickening, digestion and biosolids stabilisation/drying. Further work investigating the potential downstream impact of the increased pathogen removal has on sludge treatment and the role EPS plays on the (ir)reversible adsorption of key pathogen groups is required to better understand the public health performance of AGS systems.
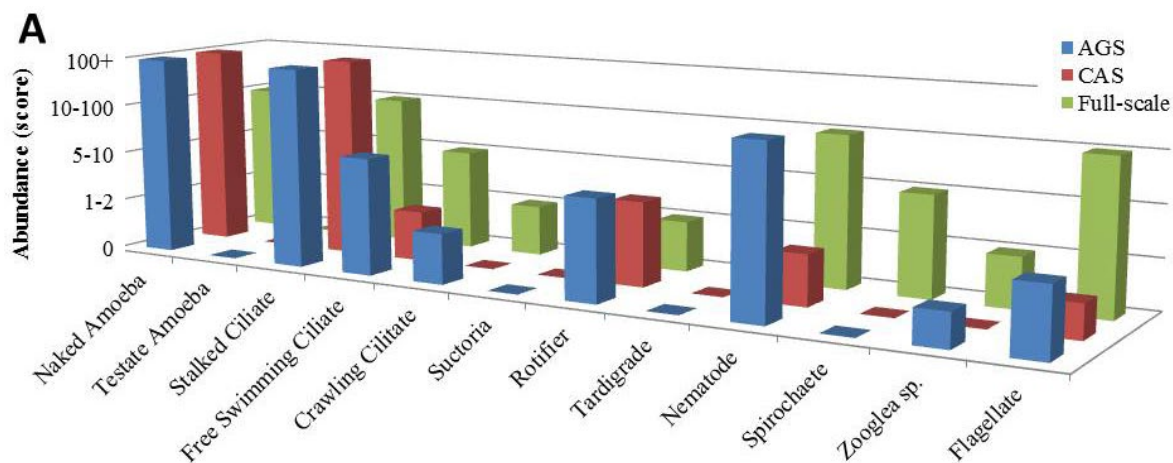
3.7 Higher Organisms

As highlighted above, pathogen removal in activated sludge processes can be achieved through several pathways, including adsorption onto the biomass or predation by resident bacteriophages, protozoa or metazoa. The predatory role played by many of these organisms in attenuating activated sludge pathogens or their surrogates has been described by Curds and Fey (1969), Kim and Unno (1996), Ng et al. (1993b) and Seviour et al. (2010). Given the potentially important role of predation in pathogen inactivation and removal in

activated sludge systems, it was important to measure and compare abundances of higher organisms in CAS and AGS, which could help explain any differences in indicator organism LRV performance. In particular, we sought to understand the abundance of those known to graze on activated sludge bacteria (i.e. amoeba, flagellates and ciliates).

Results showed that higher organism abundances remained stable throughout the course of the study, with no observable changes in the abundances of all amoeba, rotifers and Zooglea in samples from the pilot AGS and CAS and the full-scale CAS systems (Figure 8A). Dubber and Gray (2011) showed that higher organisms such as amoeba and ciliates abundances are not affected by periodic anoxic or anaerobic conditions, which is also supported by the findings of this study (Supplementary Figure 3) as such operational conditions which occur in both the AGS and CAS systems presented here and in other studies. Bray-Curtis analysis showed that there is no pronounced difference in higher organism diversity or abundance found within the AGS biomass when compared to both the CAS pilot and neighbouring full-scale SBR plant (Supplementary Figure 4). Given this similarity, the potential role for predation in AGS and CAS is similar and is therefore unlikely to explain differences seen in indicator organism LRV performances between the two systems.

In addition to the analysis of higher organisms, the presence and relative abundance of filamentous bacteria in the MLSS of AGS and CAS pilots and the full-scale SBR was investigated (Figure 8B). While filamentous bacteria may not play a direct role in the removal of microbial indicators via predation or adsorption, they do impact on biomass liquid separation performance and hence effluent water quality (Eikelboom and van Buijsen, 1993). For example, a healthy and balanced community of filamentous bacteria play an important role in floc formation and serve to catch and hold small particles during sludge settling, thereby yielding a lower turbidity effluent. Conversely, the proliferation of filaments will result in sludge bulking and elevated effluent solids. Given the relationship between effluent turbidity and effluent pathogen load, it was therefore necessary to consider differences in filamentous bacteria type and abundance between AGS and CAS. Figure 8b showed a reduction in filamentous bacteria Type 021N, Type 1851 and Beggiatoa abundance in AGS compared to CAS, however the diversity was similar. Data analysis using Bray-Curtis analysis found that the ecologies of filamentous bacteria in AGS and CAS samples were >70% similar (Supplementary Figure 4). The reduction in observable filamentous bacteria in the AGS biomass samples is consistent with previous research conducted by Tay et al. (2001) and van den Akker et al. (2015). This highlights the benefit of AGS limiting filamentous bacterial growth to facilitate superior sludge settling, however not to the extent that would be detrimental to the clarified effluent quality.
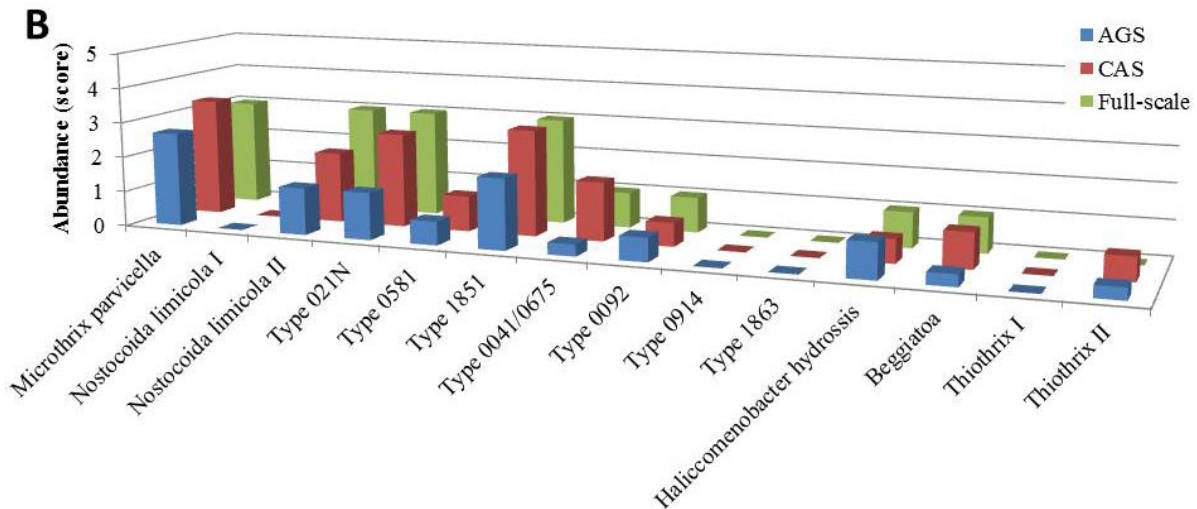
Figure 8: Comparative analysis for filamentous bacteria (A) higher organisms and (B) present in mixed liquor suspended solids collected from AGS and CAS pilot plants as well as full-scale CAS SBR. Mean abundances of organisms presented from samples (n = 3).

3.8 Implications of AGS on secondary effluent quality and water reuse

Secondary effluent samples were analysed for UVT (absorbance at 254 nm), turbidity and TSS (Table 4). TSS analysis showed that the concentration was not significantly different ($t_{(39)}$=1.51; p=0.14) between the AGS and CAS pilots. The turbidity results showed that there was a decreased turbidity of the effluent samples collected from the AGS pilot ($t_{(14)}$=0.77; p=0.46) compared to the CAS pilot. Particle size profiling (Section 2.9) was completed on secondary effluent samples from both pilot reactors as well as the full-scale SBR in order to better understand the impacts of various un-retained biomass size fractions on the final effluent quality. Analysis was conducted on samples drawn from sampling location A (Figure 1) to represent the worst case scenario, as this location collected a greater fraction of washed out MLSS. Effluent particle size profiles (Figure 9) showed a greater similarity between CAS operation (pilot and full-scale), with relatively more larger particles (≥100 μm) in the final effluent, while particles washed out of the matured AGS pilot showed a decreased abundance of particles at larger sizes (≥100 μm) and a decreased total suspended solids concentration (Table 4). This indicates that while small supracolliodal (1–100 μm) particles are persistent in both biomass samples there was a divergence in profile around 30 μm with AGS having a reduction in the presence of larger material. In addition, there was a decreased abundance of the settleable material (>100 μm) and the large reduction in large (>150 μm) particles also suggests that the retained biomass was rapidly settling granular structures as the majority of washed out material is within the conventional activated sludge size spectrum. This shows that AGS operation is unlikely to negatively impact the downstream tertiary treatment processes such as chlorination and UV disinfection which are highly sensitive to increases in suspended solids and turbidity (Chahal et al., 2016). Additionally the secondary effluent quality would not be negatively impacted during AGS start-up and mature operation.

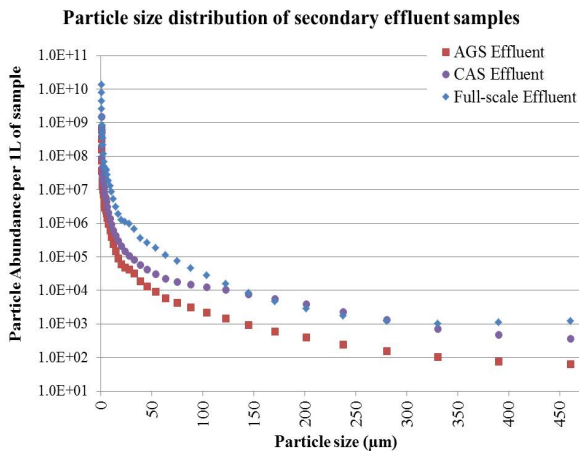**Particle size distribution of secondary effluent samples**

Figure 9: Analysis of secondary effluent particle size distribution (0.37–460.27 µm) from pilot AGS and CAS reactors alongside full-scale CAS effluent.

Table 4: Secondary effluent water quality data for AGS and CAS pilot reactors collected during the 113 days of operation (mean parameter data given ± 1 s.d.)

UVT (absorbance at 254 nm)		Turbidity (NTU)		TSS (mg/L)	
AGS	CAS	AGS	CAS	AGS	CAS
0.3 ± 0.02	0.3 ± 0.04	4.2 ± 1.6	5.2 ± 2.6	48.3 ± 18.3	58.4 ± 21.5

Results from microbial indicator monitoring have shown that AGS offers an increased capability to remove and/or retain SRC spores, *E. coli* and total coliforms. This increased removal performance has the potential to allow for a direct process saving through the reduction in the tertiary treatment requirements for water recycling applications. Nominally, as the largest increase in removal performance for AGS over CAS was identified in the SRC spores, the greatest saving would therefore be in the reduction of disinfection. Additionally, since it was shown that the transition to AGS operation did not significantly impact secondary effluent water quality, it is unlikely to require any additional treatment in terms of filtration and disinfection power/reactors.

For wastewater recycling schemes, individual treatment barriers are typically conservatively accredited based on their validated 5th percentile LRV as a worst case performance indicator. This research has shown that for AGS operation, improvements in microbial LRV capacity are likely to translate into treatment operations and capital savings for downstream disinfection processes, while still meeting regulated recycled water quality guidelines (such as health-based targets). Increase in LRV by applying AGS may suggest that tertiary UV or chlorine doses could be reduced, or depending on the end-use, additional tertiary treatment or onsite exposure controls may not be required. Results also showed that immature AGS had no detrimental impact on LRV performance when compared to CAS performance, which provides confidence that there are no adverse impacts on exceeded health-based targets during start-up which involves active washout of poor settling biomass.

4. Conclusion

The application and performance of AGS has been widely investigated for many types of sewage and various performance characteristics; however, to date no reports of AGS pathogen removal performance exist. This study showed that microbial indicator LRV performance of AGS was equivalent to CAS for bacteria and viruses and significantly better for SRC spores (protozoan pathogen surrogate). Treated effluent water quality was also maintained during the pilot start-up phase for CAS to AGS conversion, suggesting that adverse impacts on downstream disinfection processes are unlikely to be experienced. This shows that AGS is capable of meeting CAS-equivalent health-based targets for pathogen removal.

During AGS start-up (0–74 days) there was more variability in LRV performance for all indicator organisms, owing to the washout of biomass and higher TSS in the effluent; nevertheless, LRV values were still higher than that recorded in the CAS systems which suggest that start-up of AGS is unlikely to pose an unacceptable level of risk of pathogen breakthrough and performance challenge to downstream disinfection and water recycling operations. Monte Carlo simulation also showed a lower variation in indicator removal performance for the mature AGS when compared to the CAS pilot.

5. Acknowledgements

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