AUSTRALIAN MEAT PROCESSOR CORPORATION

High-Rate Aerobic Treatment Combined With Anaerobic Digestion and Anammox

Project code:	2013/4006
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Date Published:	June 2015
Published by:	Australian Meat Processor Corporation

AMPC acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Executive Summary

Australian red meat processing facilities can produce significant volumes of wastewater during slaughtering and cleaning operations. This wastewater stream is typically characterised by highly variable levels of suspended solids, organic matter and nutrient compounds, sometimes at concentrations more than four times greater than domestic sewage. It is important to treat this wastewater before discharging it into the environment or sewers. Typical treatment methods involve pre-treatment (dissolved-air flotation), followed by treatment in anaerobic lagoons to remove organic matter, and removal of biological nutrients – often by adding chemicals to remove phosphorus (P) if required. This combination of treatments creates consumes large amounts of energy, creating a substantial environmental footprint. It also uses significant amounts of carbon (C) to remove nutrients, and generates a considerable amount of sludge that is not easily degradable.

This project aimed to investigate a new system for treating meat processing wastewater – one that incorporates a high-rate sequencing batch reactor (SBR) process to remove C and nutrients; an anaerobic digestion (AD) process to stabilise the production of sludge and bioenergy (methane); and an anaerobic ammonium oxidation (anammox) process to eliminate residual nitrogen (N).

The high-rate SBR process was the most effective system, with a hydraulic retention time of half to one day and a solids retention time (SRT) of two to 2.5 days, which removed around 80% of the C and P, and 55% of the N. Biological removal of P was very efficient, mediated by a new polyphosphate-accumulating organism (PAO) called *Comamonadaceae.* The high-rate SBR process generates short sludge-age (two to four days) activated sludge, which can be digested by thermophilic, mesophilic, or two-stage temperature-phased AD processes. Four-day SRT sludge can achieve 60% degradability, and this rises to over 80% with two-day SRT sludge. The sludge dewatering liquor from thermophilic AD can also be effectively treated via anammox to almost completely remove the residual N.

Overall, this integrated high-rate system is a very compact and highly efficient treatment option for processing the wastewater created by red meat processing. It may reduce overall space requirements by more than 90% compared to current anaerobic lagoon and BNR technologies, and could achieve a considerable net energy output by reducing aerobic oxidation and increasing methane production – likely a world first in treating this type of wastewater.

Table of Contents

Glossary

Anammox	anaerobic ammonium oxidation
Bio-P removal	biological phosphorus removal
BMP	biochemical methane potential
BNR	biological nutrient removal
COD	chemical oxygen demand
DAF	dissolved-air flotation
DO	dissolved oxygen
f_d	degradability extent (degradable fraction)
FIA	flow injection analysis
FISH	fluorescent *in situ* hybridisation
FOG	fat, oil and grease
GAO	glycogen-accumulating organism
GC	gas chromatography
GHG	greenhouse gas
HRT	hydraulic retention time
k_{hyd}	apparent first-order hydrolysis rate coefficient
k_{hyd1}	k_{hyd} in the thermophilic stage of TPAD batch tests
k_{hyd2}	k_{hyd} in the mesophilic stage of TPAD batch tests
N	nitrogen
NH_4^+-N	ammonium nitrogen
NO_2^--N	nitrite nitrogen
NO_3^--N	nitrate nitrogen
P	phosphorus
PAO	polyphosphate-accumulating organism
PLC	process logic control
PO_4^{3-}-P	phosphate phosphorus
polyP	polyphosphate
SBR	sequencing batch Reactor
SCOD	soluble COD
SRT	sludge retention time
TCOD	total COD
TKN	total Kjeldahl nitrogen
TKP	total Kjeldahl phosphorus
TPAD	temperature-phased anaerobic digestion
TS	total solids
TSS	total suspended solids
VFA	volatile fatty acid
VS	volatile solids
WWTP	wastewater treatment plant

1.0 Introduction

1.1 Project Purpose and Description

This project aimed to develop an innovative technology for treating the wastewater created by red meat processing – one that achieves a high standard of wastewater treatment, including removing nutrients, while minimising the energy and footprint impacts of the process. This technology involved a high-rate aerobic mainstream treatment, before recovering energy using the high-rate sludge from the aerobic stage, and then treating the dewatered anaerobic effluent using a low-energy anaerobic ammonium oxidation (anammox) process. This is a novel alternative for red meat processing facilities that want to minimise their nutrient discharge levels, energy inputs and greenhouse gas (GHG) emissions, while also potentially creating high-quality effluent that could increase their water recycling opportunities.

1.2 Project Objectives

The main project objective of the project was to investigate a novel approach for wastewater treatment for red meat processing effluent, with the aim to maximise the carbon and nutrient removal performance while minimising the energy demand for the treatment process.

The sub-objectives are detailed as follows:

- Characterise the high-rate wastewater treatment process in terms of the nutrient removal performance and oxygen requirements
- Investigate biological phosphorus (bio-p) removal in the high-rate process and identify the bio-p removal culture
- Determine the anaerobic digestibility and biogas production of the waste-activated sludge generated
- Evaluate whether an anammox process would be suitable for treating the high-nutrient side-stream resulting from the anaerobic digestion.

A key output of this project was a set of design, operating and performance parameters for this innovative technology that could provide an economic alternative to current treatment options in situations where nutrient removal is important and/or space is limited. These parameters are an essential basis for evaluating whether the technology will be suitable and economically viable once it is implemented at full scale.

1.3 Background

1.3.1 Sources and properties of meat processing wastewater

Australian red meat processing facilities can generate large volumes of wastewater rich in organic contaminants and nutrients.[1] Although it is potentially expensive, contaminants can be removed to comply with water discharge regulations. There is also considerable potential to mitigate these costs by reducing energy and chemical inputs and maximising energy, nutrient and water recovery.

Waste and wastewater originate from a range of sources during meat processing, and can be largely divided into two streams: the green stream from stockyard washing, emptying of the animal stomachs and further processing of internal organs; the red stream from slaughter and evisceration areas, which contains blood and fats. These two streams are normally separated within the abattoir but are then combined for overall treatment (for example, in an anaerobic lagoon). The combined

wastewater properties can vary considerably across sites and even within one site, but the general characteristics are [1, 2]:

- High organic loads (represented by chemical oxygen demand (COD)) due to the presence of blood, fat, manure and undigested stomach contents
- High concentrations of fat
- High concentrations of N, P and sodium
- Fluctuating ph caused by caustic and acidic cleaning agents
- Fluctuating temperature.

1.3.2 Current status of meat processing wastewater treatment

The current treatment methods for meat processing wastewater vary greatly; however, the principal setup for wastewater treatment in the Australian red meat industry includes: primary treatment for separating solids; secondary treatment to remove dissolved and suspended organic matter that remains after primary treatment, using biological processes under aerobic or anaerobic conditions; and tertiary treatment to remove N and P, improving the quality of effluent before it is discharged.

Processes in Australia generally include dissolved-air flotation (DAF) as a primary treatment to remove fat, oil and grease (FOG) and total suspended solids (TSS). DAF effluent is then fed into the secondary treatment process, which may include anaerobic lagoons and hydraulic retention times (HRTs) of 10–20 days. Numerous lagoons can be operated in parallel or in series, increasing the effective removal of organic matter (COD). However, lagoon-based processes have a larger environmental footprint, limited or no nutrient removal, greater potential odour problems and more GHG (methane) emissions compared to more engineered digestion systems.

The tertiary treatment step usually involves BNR to remove N (typically via nitrification and denitrification), chemical precipitation to remove P, if required. However, BNR processes may require comparatively high energy inputs for aeration, external carbon sources for denitrification, and chemicals to remove P, resulting in high operating costs. More importantly, the SRT or sludge age produced in the BNR process is generally in 15–20 days, which retains sufficient slower growing nitrifiers for nitrification.[2] This in turn generates a considerable amount of long sludge-age waste-activated sludge, which has inherently low degradability under aerobic or anaerobic conditions, as inert materials from influent and recalcitrant decay products accumulate in the sludge.[3] This introduces new challenges in the subsequent sludge stabilisation process, such as poor methane production and long digester HRT, which demands large digester volumes and high capital investments.

1.3.3 Project motivation

To address these limitations in current meat processing wastewater treatment, alternative options need to:

- Treat wastewater efficiently (including removing nutrients) while ensuring low energy consumption
- Stabilise the subsequent sludge more efficiently, increasing biogas production to offset energy demands

- Reduce space and capital investment requirements, making the whole treatment process more sustainable and affordable for the meat processing industry.

One promising option is a high-rate activated sludge process that uses short HRTs and SRTs, which stimulate rapid biomass growth to remove carbon and some nutrients from the wastewater.[4] This process aims to convert most of the organics into biomass at short SRT conditions – rather than oxidising them. It dramatically reduces aeration requirements, with a corresponding lower energy demand, and can maximise the transfer of soluble and particular organics from wastewater into biomass. This biomass can then be readily digested under anaerobic conditions, producing biogas that can be used to generate energy.

In order to further achieve effective N removal in such a high-rate system, the industry has investigated new processes such as anammox[5], incorporating them into domestic treatment processes. Anammox requires very low energy input and no carbon source; it is a biological process whereby ammonium (NH_4^+) is oxidised directly to nitrogen gas using nitrite (NO_2^-) as the electron donor, as shown in Equation 1. Because it is an anaerobic process, the aeration and energy demands of the anammox process are greatly reduced compared to traditional treatment methods. It can also greatly reduce GHG emissions (CO_2 and N_2O), by using a 'shortcut' in the N cycle compared to nitrification and denitrification. Additionally, anammox is particularly suitable for treating the sludge dewatering liquor (rich in NH_4^+) generated from anaerobic sludge digestion.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

P is the second most important nutrient contained in meat processing wastewater, and must be removed before the processing plant can meet reduced discharge limits for the effluent P concentration. Recently, the focus has shifted from effectively removing P towards enhancing opportunities for downstream P recovery, due to recent increases in global P prices. This means there is strong interest in removing Bio-P in the high-rate system, because P accumulates in the bacterial cells that comprise the biological sludge and can be more readily recovered through downstream processing (such as struvite crystallisation) compared to the traditional chemical P removal. However, conventional Bio-P removal processes require a relatively longer SRT of eight to 15 days to retain the key organisms[6], which is far beyond the SRT range of less than five days currently applied in high-rate systems. Moreover, this long SRT can make it harder to maintain a stable Bio-P removal process, as some organisms (including denitrifiers and GAOs) compete with the functional P-removing organisms (PAOs) for the available C sources, resulting in a decreased PAO activity and less efficient P removal. It is important that any new Bio-P removal process can be integrated into the high-rate system to efficiently remove C and P from wastewater while also maximising the potential to recover P further downstream.

In summary, the proposed treatment train for meat processing wastewater in this project consists of three key units (as illustrated in Figure 1):

A high-rate activated sludge process that efficiently and concurrently removes C and P

Anaerobic digestion of the biomass generated in the high-rate stage, to produce energy

N removal via anammox process, with a low energy input and no carbon requirement.

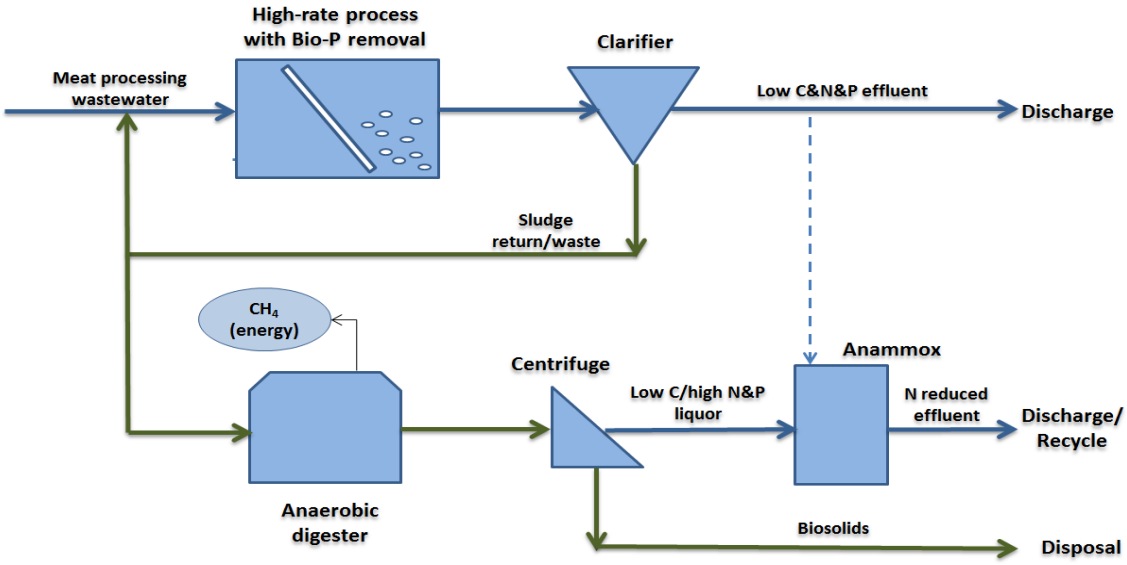

Figure 1: High-rate activated sludge process integrating anaerobic digestion and anammox

This proposed treatment system could perform better and more economically compared with current treatment technologies.

The main expected advantages are:

- Significantly reduced energy consumption – and even a considerable net energy output
- Massively reduced ghg emissions and low or no odour
- Significantly smaller land footprint.

2.0 Methodology

2.1 Characteristics of Meat Processing Wastewater

After separating DAF and solid paunch, wastewater was collected fortnightly from a large beef processing facility in South East Queensland and stored at 4°C. The wastewater was diluted with tap water to a total COD (TCOD) of 2–3 g L^{-1}, to dampen variations in the wastewater strength and composition caused by intermittently collecting wastewater from the site. Characteristics of the wastewater feed were analysed regularly, as summarised in Table 1.

Table 1: Characteristics of the wastewater collected from the facility

Parameter	Unit	Feed Wastewater Value
TCOD	g L^{-1}	2.9 ± 0.2
SCOD	g L^{-1}	1.4 ± 0.1
pH	–	7–7.8
TKN	g L^{-1}	0.1 ± 0.06
NH_4^+-N	g L^{-1}	0.07 ± 0.01
TKP	g L^{-1}	0.03 ± 0.01
PO_4^{3-}-P	g L^{-1}	0.02 ± 0.01

2.2 SBR Setup and Operation

A lab-scale SBR with a working volume of 5.3 L (Figure 2, left) operated in a temperature controlled laboratory (at 20–22°C), performing the high-rate aerobic wastewater treatment. At the commencement of the trial, the SBR was inoculated with sludge collected from a full-scale N-removal wastewater treatment plant that treats domestic wastewater in Brisbane, Australia. The SBR followed a three-hour operating cycle for eight cycles per day. Each cycle began with a 10-minute anaerobic period (feeding occurred in the first five minutes), followed by 105 minutes of aeration, 60 minutes of settling and 5 minutes of decanting (Figure 2, right). During aeration, air was provided intermittently using an on/off control system to keep the dissolved oxygen (DO) levels between 1.5 and 2mg O_2 L^{-1}. The pH was monitored but not controlled, and ranged between 7.0 and 7.8. The HRT and SRT of the SBR were maintained by discharging effluent during the decanting period, and by wasting sludge during the last 5 minutes of the aeration period, respectively.

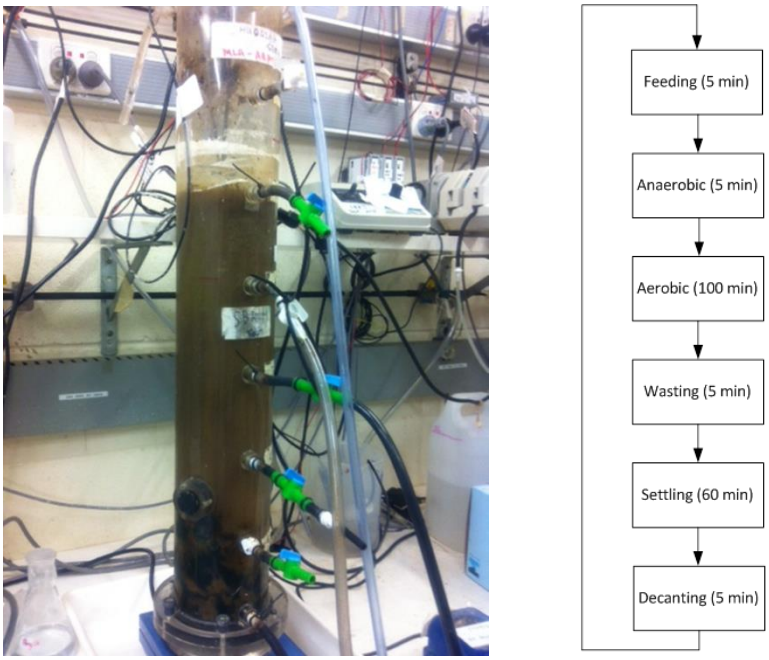

Figure 2: The high-rate SBR for treating meat processing wastewater (left) and the operating SBR cycle time (right)

The SBR operated throughout the whole research program. In the first 10 months, the SRT and HRT were altered to optimise the SBR performance, as shown in Table 2. For the rest of the operating time, the SBR operated at a constant of two days SRT and half a day HRT (considered to be the optimal conditions). To monitor the SBR performance, mixed liquor samples were taken regularly and analysed for TCOD, soluble COD (SCOD), TSS, volatile suspended solids (VSS), total Kjeldahl phosphorus (TKP), total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), nitrite-nitrogen (NO_2^--N), phosphate-phosphorus (PO_4^{3-}-P) and volatile fatty acids (VFAs).

Table 2: Summary of the SRTs and HRTs altered in the SBR to optimise the treatment performance

Operating periods	HRT (day)	Target SRT (day)	Real SRT (day)*
Start-up (55 days)	0.5	2	2.1
Period 1 (33 days)	0.5	3	2.8
Period 2 (21 days)	0.5	2	1.9
Period 3 (28 days)	0.5	4	3.8
Period 4 (27 days)	0.5	2.5	2.3
Period 5 (25 days)	1	2.5	2.4
Period 6 (27 days)	1	2	2
Period 7 (39 days)	0.5	2	2
Period 8 (15 days)	1	2	2
Period 9 (18 days)	1	1.7	1.7

**In some periods the real SRT of the SBR was slightly different than the target SRT, when taking the solids concentration of the effluent into account.*

2.3 Setup and Operation of Anaerobic Digesters

Two anaerobic digesters (0.8-litre working volume, see Figure 3) stabilised the activated sludge generated as waste from the high-rate SBR process. Both digesters operated in parallel throughout the whole research program; one at 55°C for 5–10 days of HRT (the thermophilic digester), the other at 35°C for 10 days of HRT (the mesophilic digester). At the beginning of the process, both digesters were inoculated from a full-scale anaerobic digester (35°C ± 1°C, for 20 days HRT) in the Luggage Point wastewater treatment plant (WWTP) in Brisbane.

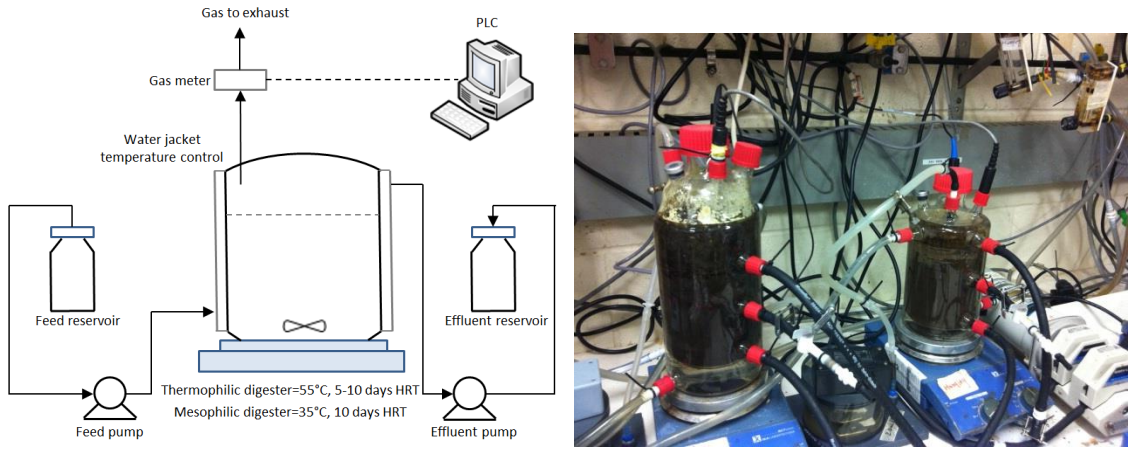

Figure 3: Setup of the anaerobic digesters for stabilising the activated sludge produced as waste from the high-rate SBR

Temperature-controlled water circulating through the water jacket maintained the temperature in the digesters, and each digester was continuously mixed using a magnetic stirring bar. Tipping-bucket gas meters measured the volume of biogas production in each digester, a process logic control (PLC) system recorded these readings online. A calibrated glass body probe measured the pH in each

digester daily, and the results were recorded online. A gas chromatography (GC) meter analysed biogas composition (specifically H_2, CH_4 and CO_2 concentration). Slurry samples were periodically collected and analysed for total solids (TS), volatile solids (VS), TCOD, SCOD, VFAs, TKN, TKP, NH_4^+-N and PO_4^{3-}P, to monitor the digester performance.

2.4 Setup and Operation of Batch Tests

2.4.1 Batch tests of anaerobic sludge digestion

Three different anaerobic sludge digestion processes — mesophilic, thermophilic and two-stage temperature-phased anaerobic digestion (TPAD) — were tested in batch conditions to assess the anaerobic degradability of the activated sludge produced as waste by the high-rate SBR process. The SBR generated the activated sludge used in the tests during Periods 1–3, corresponding to a sludge age of two, three and four days, respectively. Methane production potential and sludge degradability (using a model-based analysis of the experimental results) were the key performance indicators for anaerobic degradability.

Single-stage mesophilic and thermophilic anaerobic digestion batch tests

The single-stage mesophilic (37°C) and thermophilic (55°C) anaerobic digestion batch tests were effectively biochemical methane potential (BMP) tests based on methods described by Angelidaki et al.[7] The inoculum used in the thermophilic batch tests was harvested from a continuous 1-litre lab-scale reactor operated at 55°C for four days of HRT. The inoculum used in the mesophilic batch tests was collected from a full-scale anaerobic digester (kept at 35°C ± 1°C, for 20 days of HRT) in the Luggage Point WWTP.

Mesophilic and thermophilic batch tests were performed in 160mL non-stirred glass serum bottles (of 100-milliletre working volume). A substrate-to-inoculum ratio of approximately 0.75 (VS basis) was maintained in all tests. Bottles were flushed with high-purity nitrogen gas for three minutes (1 litre min^{-1}), sealed with a rubber stopper retained with an aluminium crimp cap, and stored in temperature-controlled incubators (±1°C). Blanks contained only inoculum and MilliQ water to measure the background methane produced from the inoculum. All tests were carried out in triplicate, and all error bars indicated 95% confidence in the average of these triplicates, based on two-tailed *t*-tests.

Two-stage TPAD-directed batch tests

The two-stage TPAD-directed batch tests consisted of two separate stages: a thermophilic pre-treatment stage (55°C, with a two-day retention time) followed by a mesophilic stage (37°C, batch to asymptote). The inoculum for each stage was the same one used in the single-stage mesophilic and thermophilic batch tests described above. The setup and operation procedures of two-stage TPAD batch tests were described previously in Ge et al.[8] Generally, the test setup in the thermophilic stage was the same as for the single-stage thermophilic batch tests (stated above) and was maintained at 55°C for two days. At the end of the thermophilic stage, a slurry sample was taken from the bottles and directly transferred into another bottle with the mesophilic inoculum, to initiate the subsequent mesophilic digestion. Blanks were also carried out in triplicate, as described above.

2.4.2 Residual BMP tests

BMP tests determined the residual methane potential from digestion residues and to assess the stability of the effluent. The setup and operation of the residual tests were the same as for the single-stage mesophilic batch tests described above. The inoculum used in the tests was collected from the same full-scale anaerobic digester in the Luggage Point WWTP. Substrates used in the tests

were digestates collected from the thermophilic digester and the mesophilic digester, respectively. The inoculum-to-substrate ratio used in the residual BMP test was 1:1 (VS basis). All tests were carried out in triplicate, and triplicate blanks were included to correct for background methane formation by the inoculum.

2.4.3 Anammox batch tests

Specific anammox activity tests evaluated the effectiveness of using anammox to treat the dewatering liquor (containing high levels of NH_4^+) from the thermophilic sludge digester digestate (after 10 days HRT at 55°C). The anammox biomass used in the tests was collected from a 50-litre anammox biofilm reactor, which operates continuously to enrich anammox culture on carriers (as shown in Figure 4, left). After researchers sampled a number of carriers from the parent reactor, they harvested biomass from the carriers by stirring the latter in nutrient medium at a low speed and then letting the biomass settle. The medium contained $CaCl_2$ (0.39 g L^{-1}), KH_2PO_4 (0.05 g L^{-1}), $MgSO_4$ (0.2 g L^{-1}), $FeSO_4$ (0.00625 g L^{-1}), EDTA (0.00625 g L^{-1}), a trace I acidic solution (1 g L^{-1}) and a trace II alkaline solution (1 g L^{-1}). The settled biomass was subsequently washed and re-suspended in new nutrient medium. The substrate used in the tests was the dewatering liquor collected by running the thermophilic digester effluent through a centrifuge.

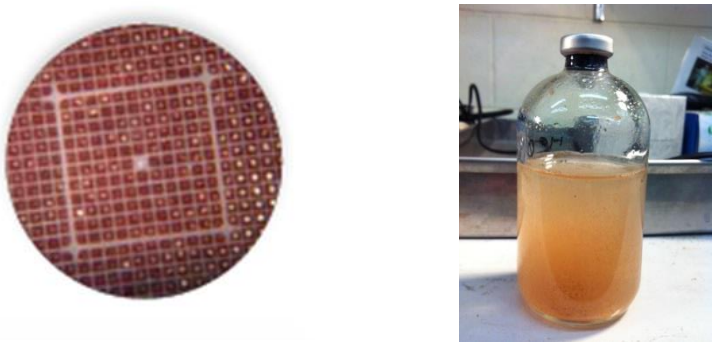

Figure 4: The carrier with enriched anammox biomass collected from the parent anammox reactor (left), and the bottle setup for the specific anammox activity tests (right).

The set-up and operation of the activity tests were based on the method described by Dapena-Mora et al.[9]. In practice, the tests were performed in 160-millilitre serum bottles (100-mL working volume). Pre-determined volumes of the anammox biomass and sludge dewatering liquor were added to achieve the initial concentrations of biomass and NH_4^+ to 1 g VSS L^{-1} and 100 mg N L^{-1}, respectively. The bottles were then sparged with pure nitrogen gas to achieve anoxic conditions, sealed with a rubber stopper retained with an aluminium crimp cap, and placed in a thermostatic shaker at 150 rpm and 30°C (Figure 4, right). The initial pH value was fixed at 7.8. NO_2^- was added step-wise to provide sufficient NO_2^- for anammox but also to maintain the NO_2^- concentration below 30 mg N L^{-1}, which was reported as an inhibition concentration for anammox.[10] A positive control assay of the activity tests was set up as described above, except with a prepared NH_4^+ stock solution added as the substrate to evaluate if the dewatering liquor had any inhibition effects.

Blanks only contained the anammox biomass and nutrient medium, and NO_2^- was still added step-wise to test the activity of denitrification, which was negligible. All the tests mentioned above were conducted in triplicate, and all error bars indicated confidence in the results in the region of 95%. Liquid samples were regularly taken from each bottle throughout the experimental period, to measure NH_4^+, NO_2^- and NO_3^-.

2.5 Analysis and Calculations

2.5.1 Chemical analysis

After collecting mixed liquor samples from the high-rate SBR and anaerobic digesters, part of each sample was filtered through Millipore filter units (with 0.45µm pore size). The filtered samples were then analysed for SCOD, inorganic nitrogen species (NH_4^+, NO_3^- and NO_2^-), PO_4^{3-} and VFAs. TSS, VSS, TS and VS were analysed using standard methods.[11] TCOD and SCOD were measured using Merck cell tests (Merck KGaA, Germany). NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, TKN and TKP were measured using a Lachat Quik-Chem 8000 Flow Injection Analyser (Lachat Instrument, Milwaukee). VFA concentrations were measured using an Agilent Technologies GC with a flame ionisation detector (FID). Effluent samples were periodically collected from the SBR to allow for biochemical oxygen demand (BOD_5 at 20°C) analysis in an external analytical services laboratory.

Biogas production and composition (H_2, CH_4, CO_2) were monitored throughout each anaerobic sludge digestion batch test. Accumulated methane production was calculated by the method described in Ge et al.[8], after subtracting blank methane production. For sludge samples collected at the start and end of each batch test, the substrate, inoculum and combined slurry samples were analysed for TCOD, SCOD, TS, VS, NH_4^+, PO_4^{3-} and VFAs. Biogas composition was analysed by a Perkin Elmer GC equipped with a thermal conductivity detector (TCD).

2.5.2 Model-based analysis

The degradability extent (f_d) and apparent first-order hydrolysis rate coefficient (k_{hyd}) were estimated as key sludge degradability properties in the anaerobic digestion tests. In the mesophilic and thermophilic anaerobic batch tests, a first-order kinetic model (Equation 2) was implemented in a modified version of Aquasim 2.1d.[12]

$$\frac{dS}{dt} = -k_{hyd}S \qquad (2)$$

Where S = degradable portion of substrate

t = time (d)

k_{hyd} = first order hydrolysis rate constant (d^{-1}).

The modified Aquasim was used to determine the two-parameter uncertainty surface for f_d and k_{hyd}, and to simulate the methane flow of the batch tests. The method for determining parameter surface was described in Batstone et al.[13] A 95% confidence limit was used, with appropriate F-values for two parameters and the number of degrees of freedom. Both correlation and non-linearity accounted for determination of the 95% confident parameter surface, which is defined by a critical value (J_{crit}), where the objective function (J) is the residual sum of squares (RSS), and J_{opt} is the optimal RSS. Under these circumstances, and with normally distributed residuals, J_{crit} can be defined as:

$$J_{crit} = J_{opt}(1+\frac{p}{N_{data}-p}F_{\alpha,P,N_{data-p}}) \quad (3)$$

where J_{crit} = critical value

N_{data} = number of measured data points

p = number of parameters

$F_{\alpha,P,N_{data-P}}$ = value of the F distribution for α, P and N_{data-p}.

Methane flow (COD basis) was used as the measured variable, with RSS as the objective function. Substrate loading was the initial condition used for model estimation and simulation.

In the analysis of TPAD-directed batch tests, the two-stage anaerobic model described in Ge et al.[8] was used to determine the 95% confidence regions for apparent hydrolysis coefficients in the thermophilic stage (k_{hyd1}) and mesophilic stage (k_{hyd2}) and f_d. In each case, these three parameters were simultaneously estimated to achieve the optimal values. The confidence region for k_{hyd1} and f_d was determined with the fixed k_{hyd2}, which used the same method as for a two-parameter system, stated above. Similarly, the confidence region for k_{hyd2} and f_d was determined by fixing k_{hyd1} at the optimal value.

2.5.3 Calculation of VS destruction

VS destruction was calculated using the Van Kleeck equation and the mass balance equation. The Van Kleeck equation (4) assumes that the amount of mineral solids is conserved during digestion[14], and uses the volatile fractions (VS/TS, termed VS_{frac}) in the inlet and outlet as references.

$$VS\ destruction\% = \frac{VS_{frac,in} - VS_{frac,out}}{VS_{frac,in} - (VS_{frac,in} \times VS_{frac,out})} \quad (4)$$

Where $VS_{frac,in}$ = volatile fraction (VS/TS) in the inlet solids

$VS_{frac,out}$ = volatile fraction (VS/TS) in the outlet solids.

The mass balance equation (5) uses VS concentrations (VS_{conc}) in the inlet and outlet, expressed as:

$$VS\ destruction\% = \frac{VS_{conc,in} - VS_{conc,out}}{VS_{conc,in}} \times 100 \quad (5)$$

Where $VS_{conc,in}$ = VS concentration of inlet

$VS_{conc,out}$ = VS concentrations of outlet.

2.6 Microbial Analysis

2.6.1 Flow cytometry analysis

Cell preparation and staining

After collection from the SBR, the fresh biomass samples were washed twice with phosphate-buffered saline (PBS) at 14,000 *g* for five minutes to remove any disturbing substances, and the

resulting pellets were resuspended in PBS. The resuspended samples were subjected to 15 sections of sonication in a water bath to disrupt flocs and then filtered through cell strainers (Becton & Dickinson (BD) Falcon, 40 µm).

The filtered biomass samples were stained with 4´, 6´- diamidino-2-phenylindole (DAPI) to separate the potential PAOs from non-PAOs based on the detection of cellular polyphosphate (polyP) at a higher DAPI concentration. The staining method was described in Günther et al.[15]; the DAPI concentration was 0.24 µm for DNA staining and 1 µm for polyP staining. The stained samples were incubated in the dark at room temperature for 60 minutes prior to flow cytometry analysis; the unstained samples served as controls. To verify reliable staining, the stained samples were examined under a microscopy to examine the blue fluorescence of DAPI-stained cells and the yellow fluorescence of DAPI-stained polyP cells.

Fluorescence-activated cell sorting (FACS)

Flow cytometry analyses were performed using a BD FACSAriaTM SORP high-speed cell sorter (USA) equipped with three lasers: an ultraviolet laser (355 nm), an argon blue laser (488 nm) and a yellow/green laser (561 nm). DAPI was activated by 60mW of ultraviolet light (355 nm). DAPI blue fluorescence was passed through a 450/50 band pass (BP) filter, and DAPI-polyP yellow fluorescence was passed through a 575/25 BP filter. To acquire the polyp-containing sub-population, the bacterial population was positioned entirely on a scale – a bivariate forward scatter (FSC) against side scatter (SSC) dot plot – with adjusting photomultiplier tube (PMT) values to position the peak on the histograms until the entire population was visible. A bivariate SP RED (575/25nm filter) against Hoechst (450/50nm filter) dot plot was also set up to place the unstained population in the lower left quadrant. The DAPI-stained samples of 0.25 µm and 1 µm were then analysed. The sort gate was set to collect the cells with higher emissions in the 575/25nm wavelength spectrum, present only in the sample stained with 1µm DAPI. The two-way aseptic sort procedure with purity set for 'single-cell duplicates allowed' was used to collect 5×10^5 polyP-containing cells into a single tube. Flow sorting of non–polyp-containing cells (10^6) was also performed in the same way, except with the sort gate set to collect the cells that had lower emissions (450/50 nm).

2.6.2 16S-rRNA Gene Amplicon Pyrosequencing

Biomass samples were taken regularly from the SBR in the start-up period and in Periods 1–4. 16S-rRNA gene Pyrotag sequencing examined the dynamic nature of the SBR microbial communities at different SRTs. A fast DNA Spin Kit for Soil (MP Biomedics, USA) was used to extract DNA from the sludge samples according to the manufacturer's protocol. Pyrotag sequencing was also used to identify the bacterial composition of the sorted cells that were rich in polyP. Alkaline lysis was used as a pre-treatment step before extracting DNA from the cells, to maximise the quantity of DNA obtained. The alkaline lysis involved mixing the sorted cells with the cell lysis solution (400 mM KOH, 10 mM EDTA, 100 mM DTT) at a 1:1 volume basis and incubating them on ice for 10 minutes, then mixing them with the neutralisation buffer (400 mM HCL, 600 mM Tris-HCL, pH 7.5) (1:1 volume basis). Subsequently, the FastDNA Spin Kit for Soil was used to extract DNA from the cell lysate.

The quantity and quality of the extracted DNA was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and agarose gel (1%, weight/volume) electrophoresis. The extracted DNA was then submitted to the Australian Centre for Ecogenomics (ACE) for 16S-rRNA gene Pyrotag sequencing on the Genome Sequencer FLX Titanium platform (Roche, USA). The primers used for Pyrotag sequencing were modifications of the 926F (5'-AAACTYAAAKGAATTGACGG-3') and 1392wR (5'-ACGGGCGGTGWGTRC-3'). Primer sequences were modified by adding Roche 454 adaptor sequences (adaptor A on the reverse primer and adaptor B on the forward) and unique 5–10 BP barcodes placed between the template-specific sequence and the A adaptor (sequences not shown).

Pyrotag sequences were processed through the ACE Pyrosequencing Pipeline[16], developed based on QIIME [17] and ACACIA [18]. The sequences with 97% similarity were then assigned to operational taxonomic units (OTUs) by CD-HIT-OTU [19, 20], aligned by Pynast [17] and assigned to the taxonomy with BlastTaxonAssigner in QIIME through Greengenes database (Oct 2011 release). The OTUs table was normalised by using Nomaliser [21].

2.6.3 Fluorescence *in situ* hybridisation (FISH) and polyP staining

FISH and polyP staining by DAPI was incompatible in a single preparation in this case; these two processes must be done sequentially. FISH was performed according to Amann.[22] Table 3 summarises the oligonucleotide probes used in this project, along with the hybridisation conditions and related references. Slides were viewed using a Zeiss LSM 510 Meta Confocal laser-scanning microscope (Zeiss, Germany) and the location of important fields noted after the image was acquired. Subsequent polyP staining was conducted by incubation with 1 µg mL^{-1} DAPI in the dark for 60 minutes.[23] The fields from which FISH images had been collected were located, and images of DAPI stains were also recorded by the Confocal microscope with filter sets that allow the emission wavelength of 450–520 nm to pass through.

Table 3: Oligonucleotide probes used for FISH.

Probe	Specificity	Sequence (5´–3´)	Formamide	Ref.
ACI208	*Acidovorax spp.*	CGCGCAAGGCCTTGC	20%	[24]
Cte	*Comamonas spp., Acidovorax spp., Hydrogenophaga spp., Aquaspirillum spp.*	TTCCATCCCCCTCTGCCG	20%	[25]
EUB338	*Most bacteria**	GCTGCCTCCCGTAGGAGT	0-50%	[26]
EUB338II		GCAGCCACCCGTAGGTGT	0-50%	[27]
EUB338III		GCTGCCACCCGTAGGTGT	0-50%	
PAO462	*Most Accumulibacter***	CCGTCATCTACWCAGGGTATTAAC	35%	[28]
PAO651		CCCTCTGCCAAACTCCAG	35%	
PAO846		GTTAGCTACGGCACTAAAAGG	35%	

**EUB338, EUB338II and EUB338III were applied simultaneously as EUBmix.*

***PAO264, PAO651, PAO846 were used in a mixture called PAOmix.*

3.0 Results for the High-Rate Wastewater Treatment

3.1 Performance of the High-Rate SBR Process

3.1.1 Carbon removal performance

Figure 4 shows the TCOD and SCOD of the SBR influent and effluent during the SBR optimisation operations after varying SRTs and HRTs. The COD removal performance is summarised in Table 4. The results after SBR optimisation are not shown in this report, as the performance was maintained at the same level.

Fifty-five days after testing commenced, the SBR achieved stable operation with approximately 87% TCOD removal and 80% SCOD removal. In the subsequent operating periods (Periods 1–4) with consistent HRT of half a day, TCOD and SCOD removal performance was maintained even while varying the SRT from two to four days (Table 4). COD removal was achieved by two processes: biomass assimilation and accumulation, and oxidation. The contribution of each process to the total COD removal was strongly influenced by SRT, as shown in Figure 5. Generally, a majority of COD (70–

80%) was removed via biomass assimilation and accumulation at 2–3 days SRT, and a smaller fraction of COD as oxidised. The fraction of oxidised COD increased to nearly 50% after four days SRT. This is consistent with oxygen consumption calculated based on the DO profiles, which progressively increased as SRT was extended from two days to four days. The HRT of the SBR was extended to one day during Period 5 (2.5 days SRT) and Period 6 (two days SRT) to further characterise the high-rate SBR process. COD removal was not significantly affected by doubling the HRT to one day, increasing the COD feed concentration during Period 8 (one day HRT, two days SRT), or decreasing the SRT to 1.7 days (Period 9). In addition, the BOD concentration of the SBR effluent was measured and found to be below 100 mg L^{-1} (Table 4), a value typically required for reuse as irrigation water.[29] Further optimising the process and potentially having a short post-aeration stage is expected to achieve an effluent BOD of less than 20mg/L, if required for environmental discharge.

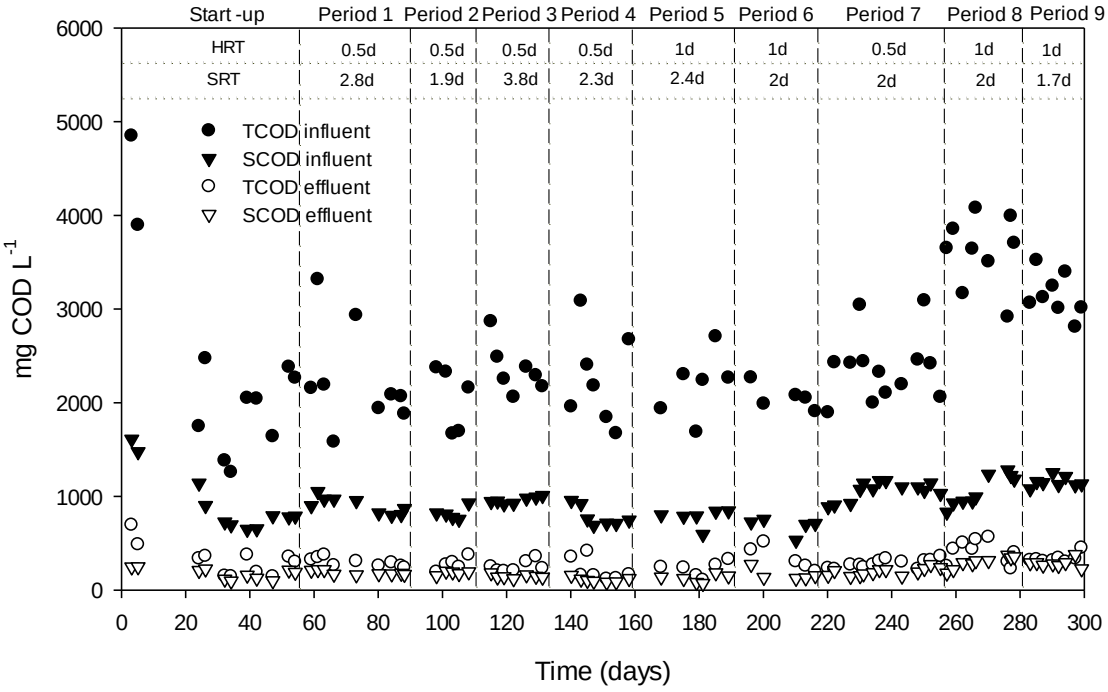

Figure 4: COD removal performance of the high-rate SBR with different operating SRTs and HRTs

Table 4: Summary of COD removal performance in the high-rate SBR with different operating SRTs and HRTs

	HRT (d)	SRT (d)	TCOD removal (%)	SCOD removal (%)	Effluent BOD (mg L^{-1})	Oxygen consumption (gO_2 d^{-1})	VSS (g L^{-1})
Start-up	0.5	2.1	86.7 ± 3.2	80.6 ± 2.2	-	6.4	3.2
Period 1	0.5	2.8	86.4 ± 2.4	79.5 ± 2.3	-	7.1	3.8
Period 2	0.5	1.9	86.2 ± 4.0	77.6 ± 2.7	85.5	6.5	3.0
Period 3	0.5	3.8	89.3 ± 2.6	84.7 ± 2.1	56.5	7.6	4.9
Period 4	0.5	2.3	90.3 ± 5.5	86.2 ± 1.9	15.7	6.8	3.1
Period 5	1	2.4	89.8 ± 3.3	84.3 ± 4.2	38.3	6.5	2.2
Period 6	1	2.0	80.9 ± 6.8	79.0 ± 8.7	-	6.1	1.7
Period 7	0.5	2.0	86.3 ± 4.8	81.4 ± 3.4	-	6.4	2.8
Period 8	1	2.0	85.1 ± 3.4	72.1 ± 3.2	-	6.7	3.1
Period 9	1	1.7	84.6 ± 3.6	78.5 ± 2.9	-	6.2	2.7

**Error margins indicate standard deviation across different measurements over each period.*

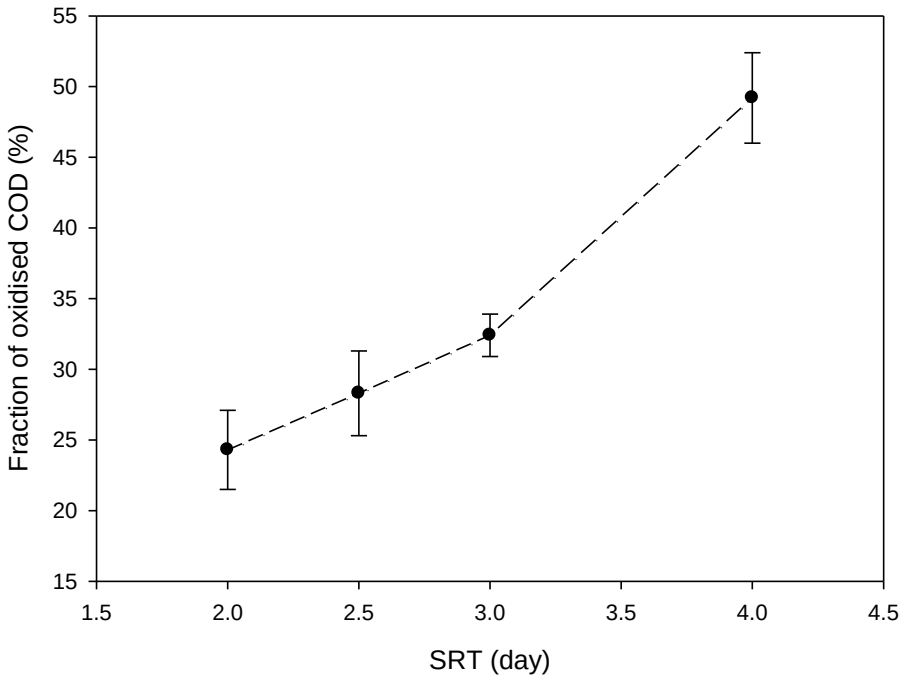

Figure 5: Impact of SRT on the fraction of oxidised COD in the total removed COD for high-rate SBR

3.1.2 Nitrogen removal performance

Figure 6 shows concentrations of total N in the SBR influent and effluent during SBR optimisation operations. In this case, the total N only contained organic N and NH_4^+; other N types (NO_2^- and NO_3^-) were negligible in comparison. Across Periods 1–9, the total N removal achieved in the SBR was approximately 56%, with more than 80% organic N removal (based on the influent organic N). The NH_4^+ removal efficiency was around 41% with 1.7–3 days SRT, with either 0.5-day or one-day HRT (Figure 7 and Table 5). When increasing the SRT to four days (0.5-day HRT) in Period 3, the effectiveness of the NH_4^+ removal substantially dropped. This was probably due to the extensive oxidation (around 50%) of the COD in the SBR (as stated above), which caused more organic (particulate) N being degraded and released NH_4^+, leading to a less effective removal of NH_4^+. The NH_4^+ removal was again suppressed during Period 7, likely due to variations in the feed composition, as reflected by the higher ratio of NH_4^+ to total N (more than 50) compared to previous periods (when it was approximately 40).

In this process, N removal is achieved by retaining organic N (mainly proteins) and growing biomass, rather than by nitrification and denitrification. This is supported by the microbial results (shown below) indicating the dominant bacterial groups in the SBR, mainly identified as aerobic heterotrophs, while known nitrifiers (such as *Nitrosomonas* and *Nitrobacter*) are virtually absent. This also eliminates the potential for generating nitrous oxide, a strong GHG, which so far has only been associated with nitrification or denitrification processes.[30]

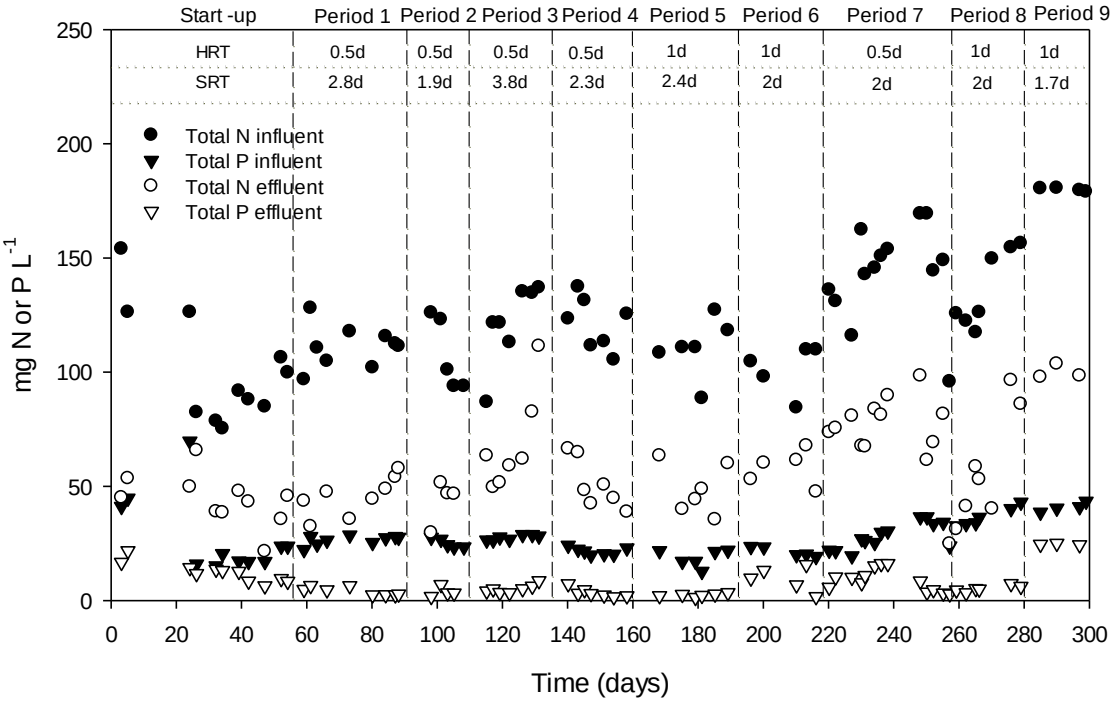

Figure 6: Removal performance of total N and P in the high-rate SBR, with different operating SRTs and HRTs

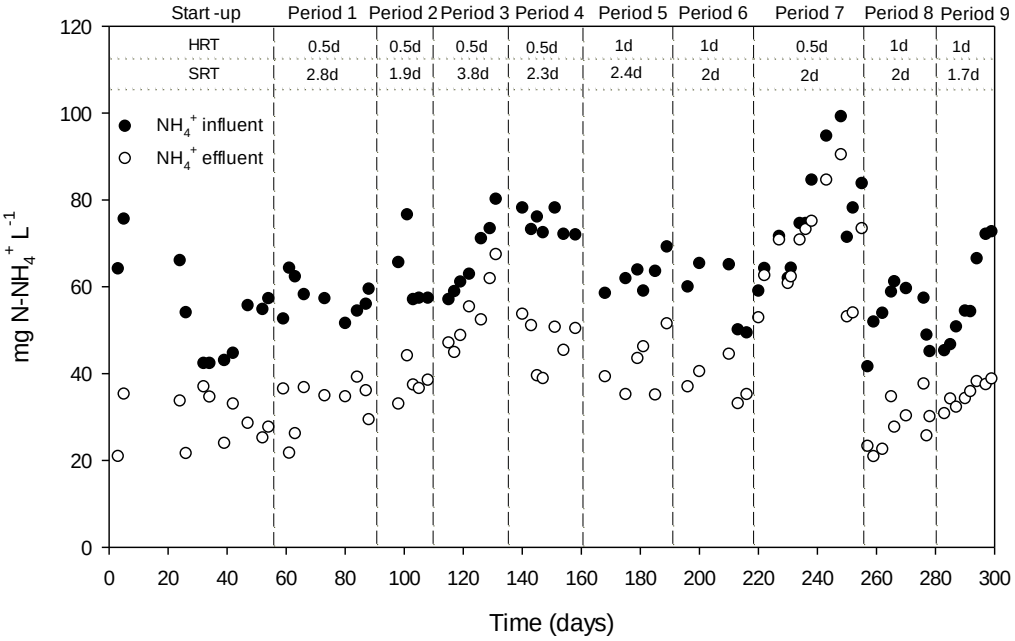

Figure 7: Ammonium (NH_4^+) removal during high-rate SBR, with different operating SRTs and HRTs

Table 5: Summary of N and P removal performance in the high-rate SBR, with different operating SRTs and HRTs

	HRT (d)	SRT (d)	Total N removal (%)	NH_4^+ removal (%)	Specific NH_4^+ removal (mg $gVSS^{-1}d^{-1}$)	Total P removal (%)	PO_4^{3-} removal (%)	Specific PO_4^{3-} removal (mg $gVSS^{-1}d^{-1}$)
Start-up	0.5	2.1	51.7 ± 6.8*	41.9 ± 5.7	10.5 ± 6.8	50.0 ± 3.5	58.7 ± 6.8	4.8 ± 2.3
Period 1	0.5	2.8	58.6 ± 9.0	42.0 ± 13.2	9.6 ± 6.6	84.6 ± 6.6	95.8 ± 7.2	8.9 ± 3.8
Period 2	0.5	1.9	59.3 ± 10.2	39.2 ± 6.9	14.3 ± 1.9	76.8 ± 1.9	90.7 ± 6.8	11.4 ± 4.2
Period 3	0.5	3.8	51.5 ± 12.5	18.8 ± 5.0	3.4 ± 5.9	85.1 ± 5.9	88.8 ± 5.9	7.2 ± 2.8
Period 4	0.5	2.3	61.5 ± 15.0	36.9 ± 7.6	14 ± 7.9	84.4 ± 7.9	91.3 ± 4.8	9.4 ± 1.9
Period 5	1	2.4	56.2 ± 11.4	38.7 ± 13.7	8.3 ± 5.3	85.3 ± 5.3	89.4 ± 6.9	5.8 ± 2.2
Period 6	1	2.0	48.9 ± 7.9	36.1 ± 3.8	10.9 ± 6.8	70.6 ± 6.8	77.6 ± 8.1	7.8 ± 3.1
Period 7	0.5	2.0	46.9 ± 8.7	8.9 ± 5.5	3.7 ± 8.1	65.1 ± 8.1	65.2 ± 10.2	9.4 ± 2.4

Period 8	1	2.0	63.6 ± 9.9	59.0 ± 13.3	7.5 ± 2.0	84.5 ± 2.0	96.1 ± 6.8	8.0 ± 2.6
Period 9	1	1.7	58.6 ± 5.8	43.1 ± 8.4	11.4 ± 5.8	72.1 ± 3.6	51.3 ± 9.6	7.0 ± 3.4

**Error margins indicate standard deviation across different measurements over each period.*

3.1.3 Phosphorus removal performance

In addition to removing N from the wastewater, the SBR consistently removed between 65% and 85% of the incoming total P, as shown in Figure 6 and Table 5 above. A high PO_4^{3-} removal was also observed at the end of the start-up period (Figure 8), and the removal efficiency was consistently more than 90% in the subsequent operating periods (Periods 1–4), where the SRT was varied from two to four days (with HRT maintained at half a day). The specific removal efficiency was highest at two days SRT, and slightly decreased after increasing the SRT to 2.5 days, and even further to three and four days (Table 5). Figure 9 shows typical PO_4^{3-} removal profiles obtained in the cycle studies performed during these periods. Based on the PO_4^{3-}-P concentrations of the raw wastewater fed into the SBR and the dilution within the SBR, the estimated PO_4^{3-}-P concentrations in the SBR bulk liquid after the feeding were much lower than the PO_4^{3-}-P concentrations measured at the end of the feeding period (Figure 9). This clearly indicated PO_4^{3-} was released to the bulk liquid during the anaerobic phase and was taken up from the liquid in the subsequent aerobic phase (a typical Bio-P removal cycle), with less than 3 mg of PO_4^{3-}-P L^{-1} remaining in the effluent. It should be noted that this is the first time (to the author's knowledge) that Bio-P removal has been achieved at such short SRTs (of 2–2.5 days). Moreover, the removal efficiency was not influenced by extending the HRT to one day (Period 5), but did drop during Periods 6–7, likely due to variations in the feed. At the end of Period 7, the Bio-P removal efficiency returned to previous levels (Figure 8), and stayed at the same level when the HRT was again extended to one day in Period 8, combined with doubling the COD feeding (to 4000 mg COD L^{-1}). Again, this indicated that the HRT did not have a substantial impact on the efficiency of Bio-P removal. Additionally, the SRT was reduced to 1.7 days to determine the absolute minimum SRT for achieving Bio-P removal. Figure 9 shows that the Bio-P removal was lost, as reflected by the lack of PO_4^{3-} release detected during the anaerobic phase, ultimately resulting in less efficient removal. This was probably because the SRT was shorter than the maximum growth rate achievable by the PAOs, so functional PAOs were washed out of the SBR.

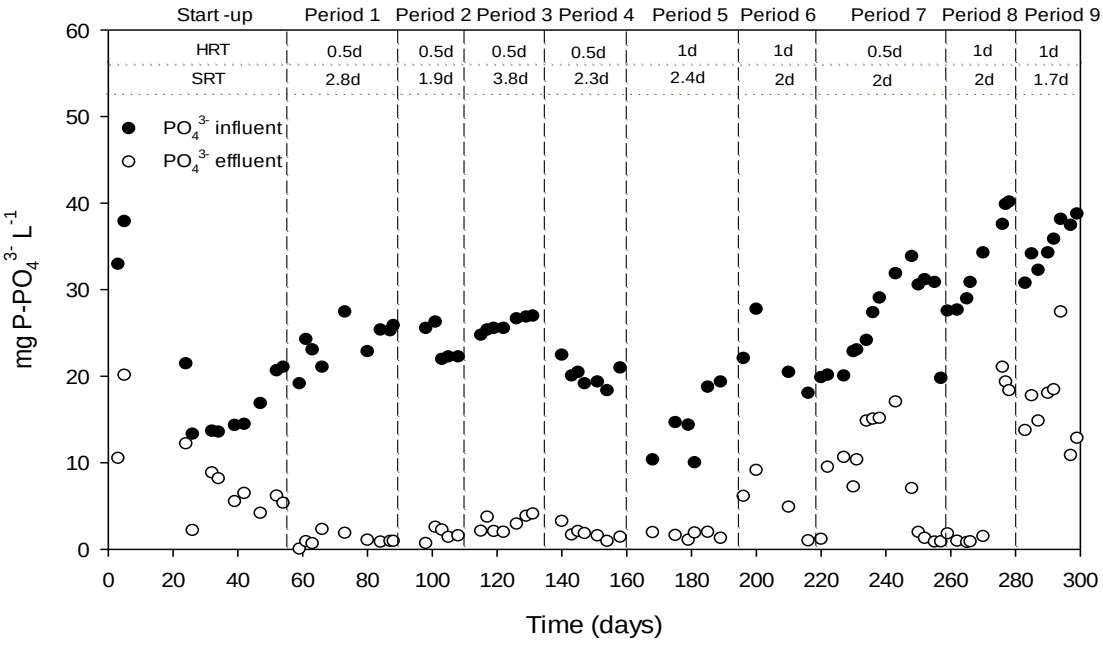

Figure 8: Phosphate (PO_4^{3-}) removal in the high-rate SBR, with different operating SRTs and HRTs

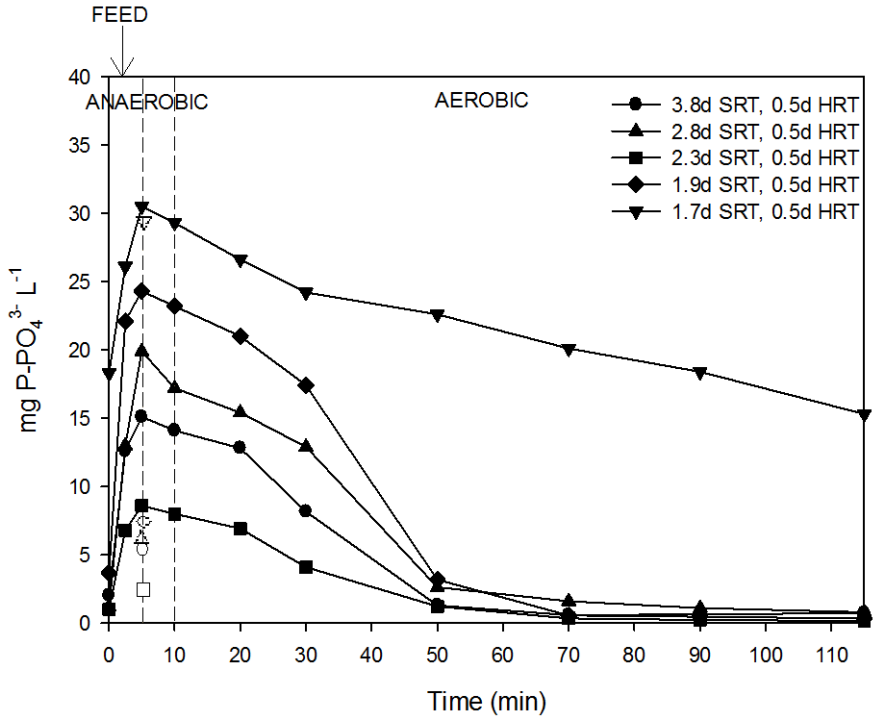

Figure 9: Phosphate (PO_4^{3-}) profiles during the SBR cycle studies, with different SRTs and HRTs. ○, Δ, □, ◊, ▽ represent the estimated PO_4^{3-} concentrations in the SBR after feeding (the calculation was based on the PO_4^{3-} concentrations of the raw wastewater fed into the SBR and the dilution within the SBR)

Overall, the high-rate SBR process removed more than 80% of the COD and P and more than 50% of the N, and the BOD level in the SBR effluent was also below the level typically required for effluent

irrigation. It suggests the effluent from the high-rate SBR process is suitable for irrigation or sewer discharge, but not for environmental discharge without further polishing for residual BOD and N removal (see the cost–benefit analysis in the following section). The decrease in COD oxidation from four days to shorter SRTs substantially reduces the required aeration demand, which significantly reduces the power requirement for this process. N (NH_4^+) and Bio-P removal was also substantially improved at 2–2.5 days compared to four days. All of these factors suggest that the high-rate SBR process is more effective at a short sludge age of 2–2.5 days. In practice, the reduced SRT and HRT would significantly reduce space requirements and construction costs, particularly compared to current treatment options that use anaerobic and aerobic lagoons. Even compared to existing BNR processes applied in the meat processing industry, the space requirement would be substantially reduced given the reduced HRT from 3–5 days to less than one day, and no anaerobic (lagoon) pre-treatment would be required. Therefore, this high-rate SBR process provides an effective, compact and energy-efficient alternative for treating meat-processing wastewater.

3.2 Microbial Community in the High-Rate SBR Process

3.2.1 Analysis of the SBR microbial communities

Figure 10 shows the composition of microbial communities in the SBR, with 2–4 days of SRT (and half-day HRT) identified by Pyrotag sequencing analysis. In general, the SBR microbial communities were relatively diverse and changed along with the progress of the SRT operation, but the population of *Comamonadaceae* consistently outcompeted other bacteria in the functionally stable SBR at different SRTs. *Comamonadaceae* has been commonly detected in activated sludge wastewater treatment processes at full and lab scales, and is capable of consuming a wide variety of organic acids, including amino acids.[31] The versatile nature of *Comamonadaceae* may have given it a competitive advantage, allowing it to become abundant in the high-rate SBR. This is because proteins (which could be fermented to amino acids) and lipids are the main components in the meat processing wastewater used in this project, and supply sufficient carbon for the growth of *Comamonadaceae* compared to other bacteria that only prefer VFAs. In this case, the family *Comamonadaceae* was primarily represented by two genera: one was predominant in all the tested periods (approximately 30%), and the other emerged after 2.5- and four-day SRTs (approximately 8%), probably induced by variations in the feed composition. Members affiliated with *Moraxellaceae* were another major bacterial group after two-, 2.5- and three-day SRTs, but plummeted after four-day SRTs, when a population surge of *Sphingomonadaceae*, *Flexibacteraceae* and *Candidatus Accumulibacter phosphatis* (within *Rhodocyclaceae*) was observed. This may be because *Moraxellaceae* is not favoured by the high NH_4^+ concentration, as the NH_4^+ removal efficiency was largely decreased after four days of SRT (as described above).

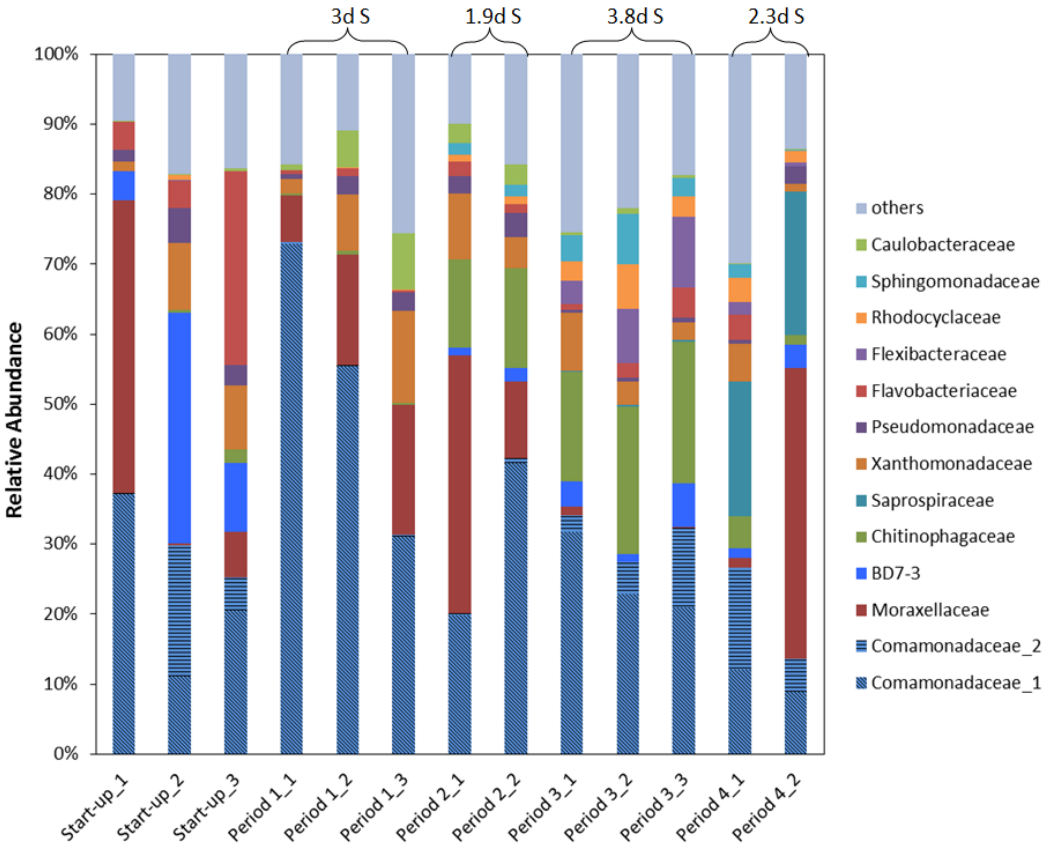

Figure 10: Microbial communities (family level, except the *BD7-3* order) identified by 16S rRNA gene Pyrotag sequencing in the SBR, in the start-up period and in Periods 1–4 (S represents SRT, *Comamonadaceae_1* and *Comamaonadaceae_2* mean different genera within the family *Comamonadaceae*)

Variations in the feed composition (organics in meat-processing wastewater) also affected the bacterial community structures in the SBR. For example, *Saprospiraceae* emerged at 2.5 days SRT, but not after an SRT of two or three days, indicating the selection pressure of feed compositions may have had more of an influence on bacterial community compositions than the SRT period. Variations in the batches of wastewater feeds (where some contained more proteins) may have supported the growth of *Saprospiraceae* rather than the rest of the mixed microbial community, as *Saprospiraceae* has been reported to be capable of hydrolysing proteins.[32] Similarly, *Chitionphagaceae* became more abundant during the two subsequent operational Periods 2–3 (two- and four-day SRTs), possibly for the same reason.

Although the SBR communities changed dynamically under different SRT conditions (over two and four days), Bio-P removal was functionally stable in the high-rate SBR. This suggests that population shifts or community fluctuations under different stress conditions (due to environmental or operational changes) maintained the functional stability of Bio-P removal. Different PAO populations appeared in the community to take the position of alternatives that may have the similar capability (maybe through different metabolic pathways), ensuring the stability of the Bio-P removal process.

3.2.2 Phylogenetic nature of the SBR communities

Figure 11 shows the phylogenetic relationship of the abundant SBR bacterial groups listed in Figure 10. Among these groups, *Comamonadaceae* was closely related to *Candidatus Accumulibacter*

phosphatis (a classic PAO clade) compared to other abundant groups. This means *Comamonadaceae* may be a strong candidate for PAOs involved in the high-rate Bio-P removal process. (Further detailed studies are presented below.) There are numerous genera within the family of *Comamonadaceae*; they may be close phylogenetic relations, but have shown different ecophysiological behaviours. For example, the genus *Curvibacter* is likely to be involved in protein hydrolysis and denitrification[33], and the genus *Malikia* has been identified as a PHA- and polyP-accumulating bacterium [34]. It indicates that different genera of *Comamonadaceae* identified in the SBR communities may play different roles in the high-rate Bio-P removal process, such as proteolysis to supply soluble C, and/or polyP accumulation.

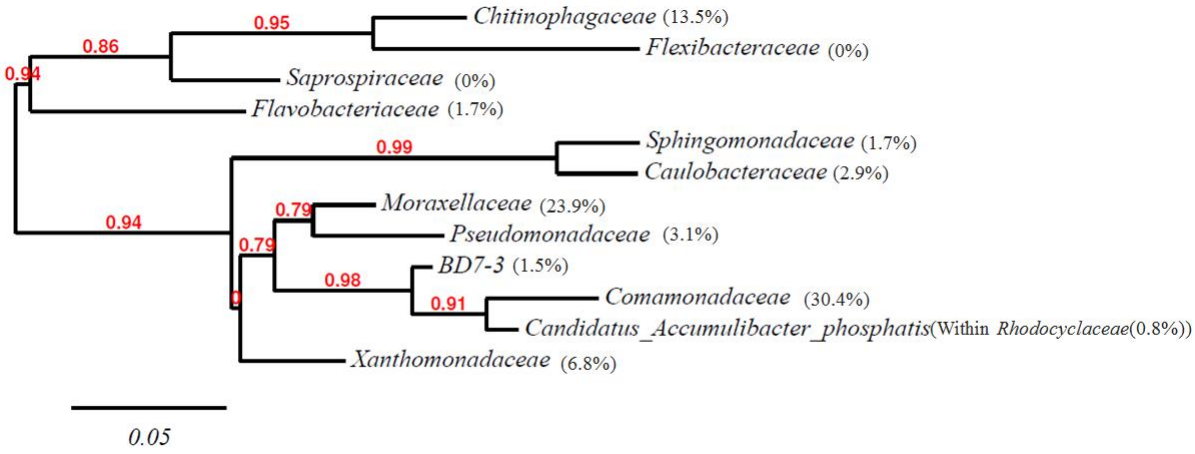

Figure 11: Maximum likelihood phylogenetic tree on 16s rRNA Pyrotag sequencing data generated according to Dereeper et al[35] (Percentage values shown in parenthesis are the relative abundance of each bacterial population at two days SRT)

3.3 Identification of Putative PAOs in the High-Rate Bio-P Removal Process

3.3.1 Separation and analysis of potential PAOs

Flow cytometry was used to physically separate the potential PAOs from the whole bacterial community in the high-rate Bio-P removal process. This was based on differences in DAPI emissions caused by the presence of polyP in PAOs and non-polyP in other bacterial cells. Three fresh biomass samples were periodically collected from the high-rate SBR (all at two-day SRT conditions) and then subjected to flow cytometry analyses. The cells containing polyP were identified in a particular region, allowing them to be separated from non-PAOs. Figure 12 shows an example of a flow cytometry plot for fluorescence emission at 450 nm, compared to 575 nm for the detected cells.

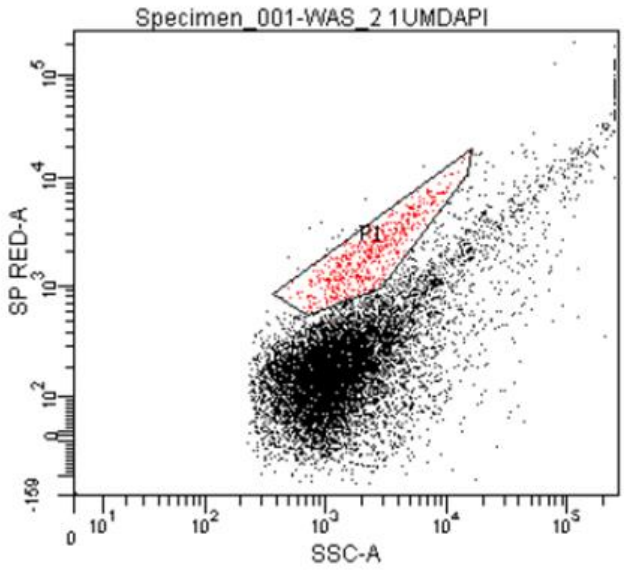

Figure 12: A flow cytometry plot of fluorescence emission at 450 nm compared to 575 nm for all detected cells, where a region (shown in red) shows cells containing polyP

The cells rich in polyP were then examined by Pyrotag sequencing analysis to identify the composition of the sorted and purified polyP-rich fractions (that is, the functional PAO communities – see Figure 13). Generally, the compositions of the polyP-rich fractions were similar; *Brucellaceae* and *Comamonadaceae* were the two most abundant populations. The *Brucellaceae* family has been detected in wastewater treatment processes before, but its functional role is unclear, especially in the Bio-P removal process. *Comamonadaceae* is a strong candidate as a potential PAO (as stated above), so further investigations were performed (see below).

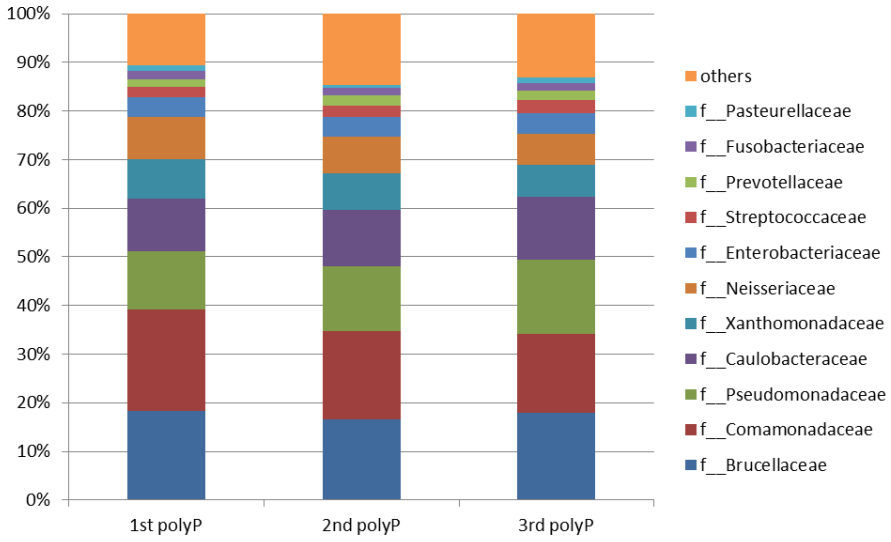

Figure 13: Compositions of the sorted polyP-rich fractions (at the family level) identified by 16S-rRNA gene Pyrotag sequencing (samples collected from the SBR at three different times)

3.3.2 FISH analysis and DAPI staining

Comamonadaceae's ability to form and store polyP (a key feature of PAOs) was further determined by using FISH analysis and DAPI staining. There is no FISH probe available to target the entire *Comamonadaceae* family, but a previously published Cte probe can target several prevalent genera in this family, including *Comamonas spp.*, *Acidovorax spp.*, *Hydrogenophaga spp.* and *Aquaspirillum spp.*[25] Bacterial cells hybridised with the Cte probe were present in all biomass samples from SRTs of 2–4-days, with three different morphotypes: small rods; small cocci formed in tetrads occurring individually or in clusters; and filaments often arranged in chains. Only the tetrad-forming bacteria were present in the DAPI stain, which showed intracellular yellowish granules suggesting polyP storage ability. Figure 14 shows an example of FISH images collected from a biomass sample of two days SRT, showing the tetrads hybridised with Cte and EUBmix probes, and DAPI-positive polyP in the tetrads. This indicates that the tetrad-forming *Comamonadaceae* defined by the Cte probe (here called tetrad-*Comamonadaceae*) were putative PAOs. The bacterial cells of *Comamonadaceae* arranged in tetrads were observed for the first time in this project; it is still unknown what factors (such as process operation and wastewater composition) cause bacterial cells to be arranged in tetrads, and what benefits can be obtained from this specific arrangement. There is a need for future studies to better understand the key factors affecting *Comamonadaceae* morphotypes and the relation of these with their phenotypes.

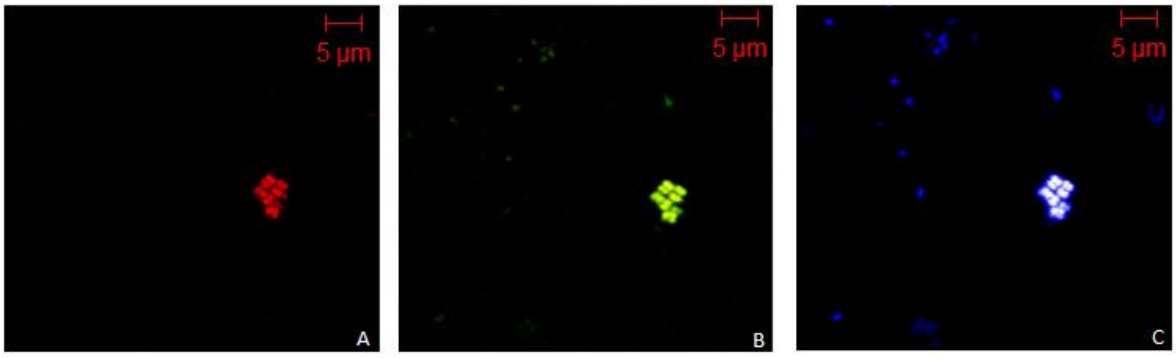

Figure 14: FISH and DAPI staining images of tetrad-forming bacteria in the high-rate SBR after two days SRT. FISH image A (left) shows tetrad-forming bacteria hybridising with the Cte bacterial probe (in red). FISH image B (middle) shows tetrad-forming bacteria hybridising with the EUBmix bacterial probes (green) and Cte probes (red). The overlay of red and green appears as yellow. C (right) is a microautoradiography image showing tetrad-forming bacteria that contain polyphosphate stained by DAPI. Polyphosphate emits a bright yellow colour and the other cells emit a blue colour. The overlay of yellow and blue appears as bright white.

3.4 Summary

The high-rate SBR activated sludge process can effectively treat red meat processing wastewater, removing around 80% of the COD and P, and 55% of the N, while generating an effluent suitable for irrigation or sewer discharge. The devised process is compact, requiring an HRT of half to one day, and 2–2.5 days SRT. Varying SRTs in the high-rate SBR had a strong impact on the composition of bacterial communities, but the population of *Comamonadaceae* was consistently dominant in the functionally stable SBR after 2–4 days of SRT. Bacterial cells of *Comamonadaceae* (defined by Cte probe) arranged in tetrads contained positive DAPI-stained polyP, indicating the high-rate Bio-P removal from meat processing wastewater at very short sludge ages was mediated by new PAO clade, *Comamonadaceae*.

4.0 Results for Anaerobic Digestion

4.1 Batch Anaerobic Digestion Tests

The sludge generated from the high-rate SBR during Periods 1–3 (corresponding to a sludge age of two, three and four days, respectively) was stabilised by three different anaerobic sludge treatment processes: mesophilic, thermophilic and two-stage TPAD treatment. These three processes were tested in batch conditions to assess the degradability of the anaerobic sludge. Figure 15 shows an example of cumulative methane production from three anaerobic digestion processes using the two-day SRT sludge. It indicates methane production over time from the digestion tests, and was continuous from the first stage to the second stage during the TPAD process.

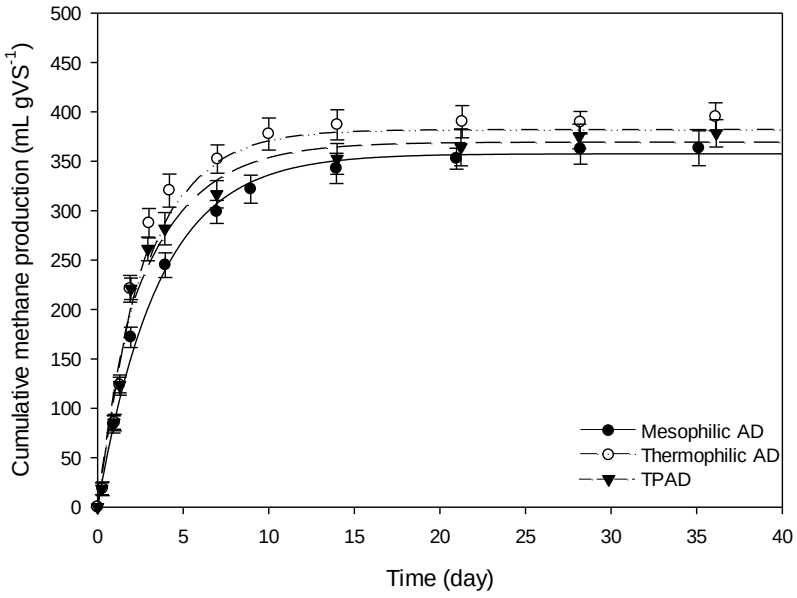

Figure 15: Cumulative methane production from mesophilic and thermophilic anaerobic digestion batch tests, and TPAD batch tests with a model fitted for two-day SRT sludge (error bars are at 95% confidence based on triplicate batch tests)

Table 6 summarises the degradability analysis results; Appendix I shows the confidence regions of k_{hyd} and f_d for each sludge digestion test. For two-day SRT sludge, the three anaerobic processes achieved a statistically similar degradation extent (f_d, approximately 84%) and hydrolysis rate (k_{hyd}, approximately 0.29 d^{-1}). For the sludge with three and four days of SRT, the degradability extents were also similar in the three anaerobic processes, reaching around 73% for the three-day SRT sludge and approximately 63% for the four-day SRT sludge. However, the hydrolysis rates were significantly improved in the thermophilic process; hydrolysis was 20–30% faster compared to the TPAD process and up to 90% faster than the mesophilic process (Table 6). Table 6 shows estimated potential methane production from each sludge digestion test, consistent with the observed cumulative methane productions from the batch tests (see Figure 15).

Table 6: Estimates of apparent hydrolysis rate coefficients (k_{hyd}, d^{-1}), degradability extents (f_d) and methane potentials (B_0, mL CH_4 gVS^{-1}) in three anaerobic digestion processes for the waste activated sludge with two, three and four days SRT

		2 days SRT	3 days SRT	4 days SRT
Single-stage mesophilic AD	k_{hyd}	0.25 ± 0.03[a]	0.22 ± 0.04	0.19 ± 0.02
	f_d	0.82 ± 0.03	0.71 ± 0.04	0.63 ± 0.03
	B_0	352.2 ± 11.2	332.4 ± 19.7	306.4 ± 12.6
Single-stage thermophilic AD	k_{hyd}	0.36 ± 0.07	0.42 ± 0.06	0.33 ± 0.06
	f_d	0.88 ± 0.05	0.71 ± 0.03	0.62 ± 0.03
	B_0	378.0 ± 19.8	332.4 ± 13.1	301.6 ± 15.6
TPAD	k_{hyd1}[b]	0.24 ± 0.02	0.28 ± 0.04	0.21 ± 0.02
	k_{hyd2}[c]	0.29 ± 0.1	0.12 ± 0.05	0.13 ± 0.05
	f_d	0.86 ± 0.06	0.79 ± 0.04	0.65 ± 0.03
	B_0	369.4 ± 23.6	340.8 ± 12.8	316.2 ± 12.2

[a]*Error margins indicate uncorrelated linear estimates of parameter uncertainty at a 95% confidence level.*

[b]*Apparent hydrolysis coefficient in the first thermophilic stage in TPAD.*

[c]*Apparent hydrolysis coefficient in the second mesophilic stage in TPAD.*

The extent of sludge degradability was greatly affected by the age of the sludge, but not by the type of anaerobic digestion applied for the range of SRTs tested in this project. The thermophilic process, and to a lesser extent the TPAD process, enhanced the hydrolysis rates for the three- and four-day SRT sludge, but not the two-day SRT sludge, probably due to its inherent rapid degradation characteristics, which are similar to primary sludge (produced by primary sedimentation processes). This increase in degradation rate is consistent with reports in literature[36, 37], which found that the sludge hydrolysis or solubilisation rate (but not the extent) depended on the temperature, showing higher rates at thermophilic temperatures than in the mesophilic range. This means that the thermophilic process can be used for a lower HRT in the digester or to intensify an existing process. In practical terms, if a long SRT process was changed to a short SRT aerobic process, any existing anaerobic digestion could be simultaneously modified to TPAD or thermophilic operation, treating the higher sludge input while still achieving a lower amount of final residues. Retrofitting a conventional process in this way would achieve a number of cost savings through reduced energy consumption and increased biogas yield, while reducing sludge volumes, since organics would be destroyed in the anaerobic digester instead of during the aerobic process.

Another concern of the anaerobic digestion process is the expected high NH_4^+ and PO_4^{3-} release, as the majority of N and P are removed into the sludge during the high-rate SBR process. A mass balance calculation for NH_4^+ and PO_4^{3-} in the anaerobic batch tests showed that approximately 500–700 mg L^{-1} NH_4^+ and 100–150 mg L^{-1} PO_4^{3-} could be released during sludge digestion. As these nutrients are in a concentrated stream, there is a good potential for nutrient recovery through struvite crystallisation and low-energy N removal during the anammox process.

4.2 Continuous Anaerobic Digesters

Two continuous anaerobic digesters supplemented testing of the thermophilic and mesophilic anaerobic processes, and provided independently derived measures for stabilisation effectiveness of the waste activated sludge generated from the high-rate SBR (with two-day SRT). The VS destruction is a key performance indicator used to show the amount of organic material converted to methane. Table 7 summaries the average VS destruction in the two digesters under different operating conditions, and Appendix II shows the VS destruction results in a time series, based on mass balance and Van Kleeck equations. VS destruction determined using the Van Kleeck equation (4) was consistent with VS destruction determined using the mass balance equation (5), which confirms that systematic sampling errors and/or unexpected behaviours were minimal. It should also be noted that both digesters were operated throughout the research program, but the performance results after varying the digester HRTs are not shown in this report, as the performance was maintained at the same level.

The thermophilic digester at 55°C with five-day HRT achieved a similar VS destruction as the mesophilic digester (35°C, 10-day HRT), which is greater than the legislated level (38%) for digestion performance implemented by the US EPA[38], and most Australian legislation. Increasing the HRT in the thermophilic digester to eight days improved VS destruction from 52% to 60%. A further increase of VS destruction to 68% was observed when the HRT in the thermophilic digester was increased to 10 days. Statistical analysis (student's *t*-test, α=0.05) confirmed that VS destruction in the thermophilic digester using longer HRTs (8–10 days) was significantly greater than that achieved in the mesophilic digester. In practice, the higher VS destruction achieved in the thermophilic digester translates to better sludge dewaterability [39] and lower overall disposal costs, which are generally weight-based. The thermophilic treatment also allows for much more hygienic output and pathogen removal compared to the mesophilic treatment.

Table 7: Summary of the performance achieved by anaerobic digesters

Digester	Operating conditions	VS destruction
Thermophilic digester	55°C, 5-day HRT	52 ± 3%
	55°C, 8-day HRT	60 ± 2%
	55°C, 10-day HRT	68 ± 4%
Mesophilic digester	35°C, 10-day HRT	50 ± 2%

In addition, the effluents from the thermophilic and the mesophilic digesters were subjected to residual BMP tests to further assess the digestion processes. Figure 16 shows an example of the digestion performance in the two digesters, which consists of three parts: VS destruction in the digester, degradable methane from the effluent, and the non-degradable recalcitrant materials. As indicated in the figure, the residual methane potential of the effluent from the thermophilic digester (55°C, 10-day HRT) and the mesophilic digester (35°C, 10-day HRT) was relatively low (less than 100 mL per VS added), which meets the disposal requirement for anaerobic digestate.

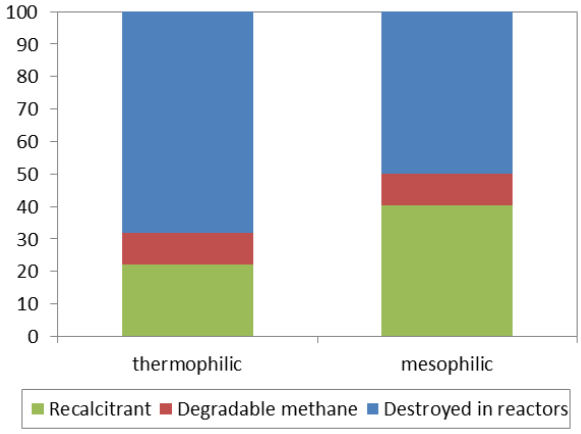

Figure 16: A summary of digestion processes in the thermophilic (55°C, 10-day HRT) and mesophilic (35°C, 10-day HRT) digesters

4.3 Summary

The short sludge age (two- to four-day) activated sludge produced from the high-rate SBR process can be successfully digested through thermophilic, mesophilic or TPAD processes; 60% degradability is achieved with four-day SRT sludge, rising to over 80% with four-day sludge. Thermophilic and TPAD processes enhanced the degradation rate of the sludge, but not the extent achieved by the mesophilic process. The effectiveness of sludge stabilisation was further tested in the continuous thermophilic (55°C) and mesophilic (35°C) digesters. VS destruction in the thermophilic digester was 20–36% higher than in the mesophilic digester, although the thermophilic digester used a five-day HRT. Using thermophilic digestion for 8–10 days HRT is shown to achieve 60–70% VS destruction for the two-day SRT sludge. This is very high for such a short HRT and close to the maximum degradability achieved in the BMP tests. The effluent from the both digesters showed the relatively low residual methane potential, indicating that the anaerobic digestate is suitable for disposal.

5.0 Results for Anammox N-Removal Testing

5.1 Anammox Batch Tests

In conventional nitrogen removal wastewater treatment plants using anaerobic sludge digestion, the recirculation of the return liquors from biosolid dewatering adds an extra 15–20% to the N load of the main stream.[40] Given the high fraction of N diverted into the sludge stream in this high-rate aerobic process, and the high level of anaerobic degradation achieved, the dewatering stream could contribute around 40% of the incoming N load back to the main stream. As such, separate treatment of this NH_4^+-rich digester supernatant can significantly reduce the N load of the activated sludge system, improving the N content of the effluent water. Compared to conventional nitrification and denitrification, anammox is an emerging option for eliminating N from wastewater with a low COD/N ratio (such as sludge dewatering liquor) for very low operating costs (due to the reduced oxygen requirements and by removing the need for organic carbon).

In this project, specific anammox activity tests evaluated the effectiveness of anammox-treating the sludge dewatering liquor from the thermophilic anaerobic digester (55°C, 10-day HRT). NO_2^- was added externally through the successive feedings, maintaining the NO_2^- level below the inhibition level. As shown in Figure 17, after each NO_2^- addition, a complete NO_2^- consumption was observed within approximately half a day and a portion of NH_4^+ then removed (as shown in Figure 18). After the five successive additions of NO_2^-, NH_4^+ was removed to a low level (less than 8 mg N L^{-1}), resulting

a total N removal rate of approximately 75 mg N L^{-1} day^{-1}. The average ratio of NH_4^+ and NO_2^- degradation was around 1.3 and is similar to the stoichiometric ratio given in Equation (1) (1:1.32). The production of NO_3^- in the tests was also monitored and the production rate was close to the theoretical value (data not shown).

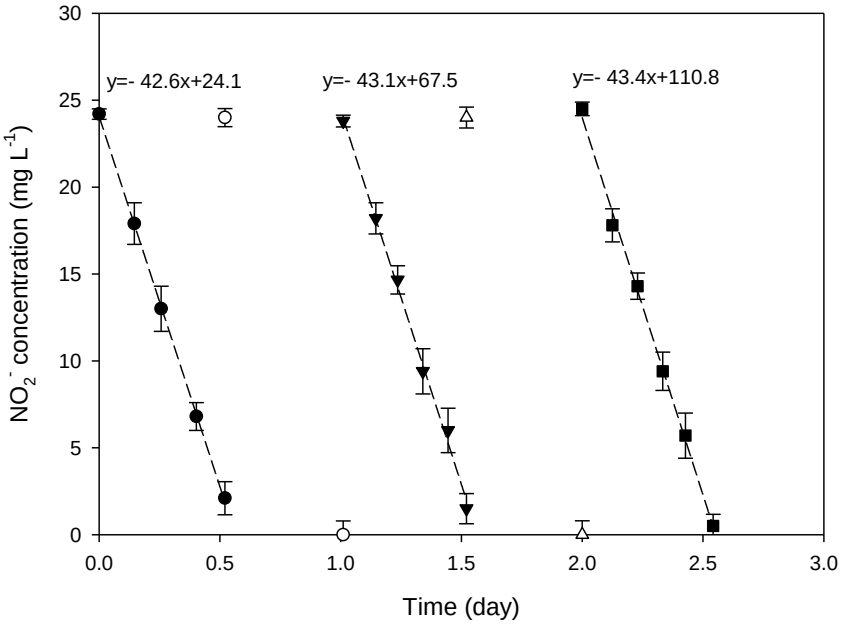

Figure 17: Nitrite (NO_2^-) removal profiles after each NO_2^- addition of approximately 25 mg N L^{-1} in the specific anammox activity tests

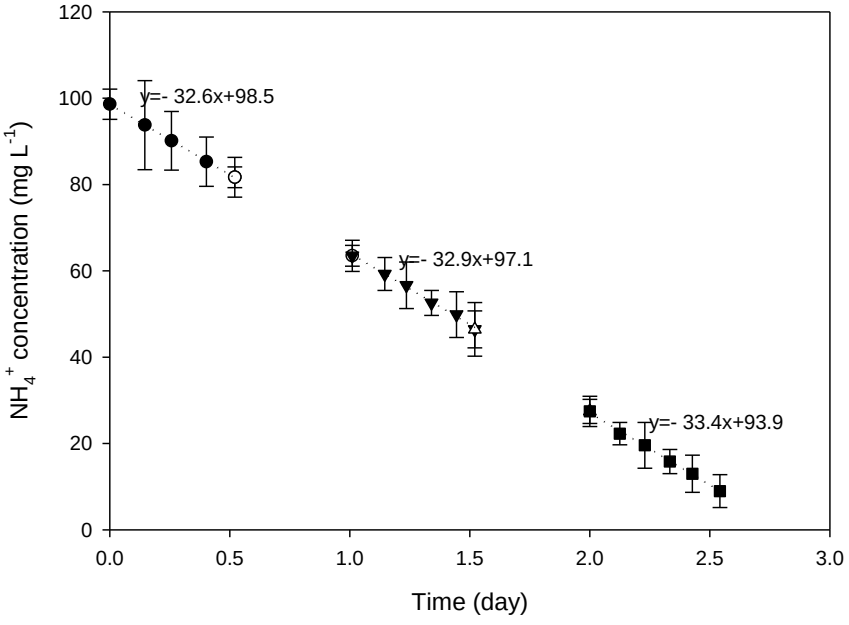

Figure 18: Ammonium (NH_4^+) removal profiles after each NO_2^- addition of approximately 25 mg N L^{-1} in the specific anammox activity tests

Another assay of the activity tests contained the anammox biomass and nutrient medium without the sludge dewatering liquor. Both NO_2^- and NH_4^+ were externally added as in the tests shown above. The average NH_4^+ and NO_2^- degradation and the NO_3^- production yielded a ratio of 1:1.35:0.33, similar to the ratio obtained in the activity tests shown above. This indicates that the real sludge dewatering liquor did not have an inhibiting or toxic effect on the anammox biomass.

It should be noted that the actual amount of anammox biomass used in the tests was less than 1 g VSS L^{-1}, as the biomass collected from the parent anammox reactor contained some NH_4^+ oxidising bacteria (AOB) (converting a part of NH_4^+ to NO_2^- for anammox). It therefore can be expected that the total N removal rate could be substantially enhanced if the anammox biomass was increased to the level normally seen in full-scale plants.

5.2 Summary

Specific anammox activity tests demonstrated that anammox can effectively remove NH_4^+ from the sludge dewatering liquor produced in the thermophilic anaerobic digester, with a total N removal rate of approximately 75 mg N L^{-1} day^{-1}. It is expected that the removal rate could be substantially enhanced by increasing the anammox biomass to the level normally seen in full-scale plants.

6.0 Cost–Benefit Analysis

This section includes a basic assessment of the integrated high-rate system developed and investigated in this project as it relates to the Australian red meat processing industry, compared to the more conventional system of anaerobic lagoons followed by an SBR-based nutrient removal process. The energy and heat balances and the space estimations are based on experimental results and assumptions sourced from existing literature; as such, results may vary for specific applications, and will need to be verified with more detailed evaluation of the system design and operational parameters. The costing information in this analysis is based on estimates of the order of magnitude; it is not intended as a detailed feasibility analysis.

6.1 Basis for the Analysis

The integrated high-rate system evaluated in this project (as illustrated in Figure 1) includes the high-rate SBR process that was shown to be most effective with two-day SRT and half-day HRT (as stated in the results section). The waste activated sludge generated from the SBR was thickened to 4% solids and treated in a thermophilic anaerobic digester (for 55°C and 10 days HRT), where approximately 70% of the organic contents were converted to biogas. The stabilised solids stream was dewatered by centrifuging and the solids cake was transported for land application, although the cost of transport and land application were not included in this analysis. The effluent streams from the high-rate SBR and the sludge dewatering liquor were combined for further treatment using anammox to remove the residual N. The analysis is based on 1 ML d^{-1} wastewater influent flow inputs, and other inputs are summarised in Table 8.

Table 8: Summary of the inputs used in cost-benefit analysis of the integrated high-rate system.

Input	Value
Wastewater volume	1,000 kL d^{-1}
COD in wastewater influent	3,000 mg L^{-1}
TSS in wastewater influent	2,000 mg L^{-1}
Total influent N	100 mgN L^{-1}
Total influent P	30 mgP L^{-1}
COD oxidation extent (in the high-rate SBR)	23%
COD removal efficiency (in the high-rate SBR)	88%
Lift pumps	10 kWh ML^{-1}
Aerators	1 kWh $kgCOD^{-1}$
Secondary thickening	0.05 kWh $kgDS^{-1}$
Wastewater and sludge temperature	20°C
TS concentration after secondary thickening	4%
VS destruction	70%
Heater efficiency for digesters	95%
Co-generation electrical efficiency	35%
Co-generation heat efficiency	50%
Digester mixing and pumping	0.18 kWh $kL^{-1}d^{-1}$
Sludge concentration after centrifuging	18%
Centrifuge	0.3 kWh $kgDS^{-1}$
Anammox loading rate	0.7 kgN kgL^{-1} d^{-1}
Anammox air requirements	1.2 kWh kgN^{-1}

P recovery using struvite crystallisation ($NH_4MgPO_4 \bullet 6H_2O$) is an emerging technology option and could be integrated into the high-rate system (after the centrifuge of the anaerobic digester effluent). However, the analysis of the costs and value recovery exceeds the scope of this project. Trade waste and discharge fees and the cost of irrigation are also not included in the analysis.

6.2 Evaluation of Energy and Heat Balances in the Integrated System

The majority of energy demand in the high-rate SBR process is from aeration and wastewater pumping, which depends on wastewater influent flow, efficient COD removal and the extent of COD oxidation.[41, 42] The main energy demand during anaerobic digestion is for pumping and mixing sludge; aeration is the main energy-consuming part of the anammox process. Other elements of the energy demand for the integrated system are from the secondary thickening of the sludge produced by the high-rate SBR, and from centrifuging the anaerobic digestion effluent for dewatering.

The main source of energy in the integrated high-rate system is the methane produced from the anaerobic digester. Methane can be used to produce electricity in a co-generation engine.[43] The waste heat from the co-generation engine can be used as the main source of heat for the anaerobic digester (see the heat balance analysis below). The potential methane production from the anaerobic digester was determined based on the VS destruction achieved in the thermophilic anaerobic digester. The energy balance was evaluated by comparing the potential energy production by the methane yield against the required energy demand for the integrated high-rate system as a whole. The results (see Table 9) indicate that in fact a net electric power output can be gained from the integrated high-rate system, achieving an energy self-sufficiency of around 170%.

Table 9: Summary of the energy input and output of the integrated high-rate system.

	Process	Sources of energy demand	kWh d^{-1}
Energy input	High-rate SBR	Aeration	607
		Wastewater pumping	10
		Secondary thickening	77
	Anaerobic digestion	Sludge mixing and pumping	69.3
		Sludge centrifuging	234
	Anammox	Aeration	327
			Total: 1,119
Energy output	Electricity recovery from methane produced in anaerobic digestion		**1,920**
Net energy gain			**801**

The heat balance in this case was assessed in the same way as the energy balance. In the integrated high-rate system the only heat demand is from the anaerobic digester, where the feed sludge needs to be heated up from ambient temperature to the required digestion temperature, and the heat lost from the digester boundary and through the piping system needs to be compensated for.[37] Generally, the heat loss in the piping can be negated by appropriate construction, and the heat loss from the digester boundary is assumed as 20% of the total heat demand.[43] The potential heat production was also determined according to the potential methane production. Table 10 shows the results of the heat balance, indicating the potential heat production can fully offset the heat

requirements for the thermophilic anaerobic digestion, with a net heat generation after electricity co-generation and feed heating.

Table 10: Summary of the heat input and output of the integrated high-rate system.

	Process	Sources of heat demand	MJ d^{-1}
Heating energy input	Anaerobic digestion	Raising the temperature of the feed sludge	5,935
		Heat losses	1,483
			Total: 7,418
Heating energy output	Heat recovery from methane produced in anaerobic digestion		**9,872**
Net heating energy gain			**2,454**

6.3 Estimation of Space Requirements for the Integrated System

A semi-quantitative estimation of the reactor sizes in the integrated high-rate system was based on the experimental results obtained in this project. Preliminary treatment processes (such as screening and DAF) are not included in the estimation, but are expected to continue unchanged. As shown in Table 11, the estimated reactor volume of the integrated high-rate system is more than 90% smaller than the most basic conventional treatment systems, which do not include anaerobic sludge digestion and tertiary treatment. This drastic reduction in reactor size is expected to bring down construction costs, and reduced energy demands will achieve lower operating costs compared to a fully aerobic or even a combined anaerobic–aerobic process. The detailed analysis of capital costs and operating costs for the integrated high-rate system is presented in AMPC project A.ENV.0162. Generally, the estimated capital cost is $4,776,000, including the vessels for the SBR, the anaerobic digester and the anammox reactor, a co-generation unit, installation and ancillaries, and engineering costs. The operating costs are estimated based on current pricing, including electricity at $0.1 kWh^{-1}, personnel at $80,000 per full time–equivalent worker, and maintenance of 2–4% of initial capital per annum. Value recovery is based on co-generation efficiency of 0.35 and $0.1 kWh^{-1}, which corresponds to a gas value of $10 GJ^{-1}. Therefore, the total annual operating expenses are estimated at $266,000. However, the following offsets need to be considered in this assessment: (a) the electricity generation from co-generation brings an annual income of $492,000; (b) using renewable energy brings renewable energy credits worth $172,000 pa (based on mid-2014 renewable energy credit situation, however the Renewable Energy Target was under Federal Government review at the time of writing). Therefore, the overall process is expected to generate a net income in the order of $400,000 per year, which is an excellent result compared to the net operating expenses of conventional wastewater treatment systems.

Table 11: Summary of the estimated reactor sizes for the integrated high-rate system, compared to the conventional system

	Process	Operational parameters	Estimated size for 1 ML d^{-1} wastewater flow
Current treatment system	Screening/DAF	–	–
	Anaerobic ponds	10–20 days HRT	10–20 ML
	SBR-based BNR process	2–5 days HRT/10–20 days SRT	2–5 ML
			Total: 15–25 ML
Integrated high-rate system	Screening/DAF	–	–
	High-rate SBR	half-day HRT/2 days SRT	0.5 ML
	Thermophilic anaerobic digester	10 days HRT	0.1 ML
	Anammox	0.5 kgN $kL^{-1}d^{-1}$ (loading rate)	0.15 ML
			Total: approx. 1 ML

7.0 Conclusions

Australian red meat processing facilities can generate large volumes of wastewater that can require treatment to remove organic and nutrient contaminants in order to comply with water discharge regulations. This project investigated a new wastewater treatment system for red meat processing effluent, incorporating three key elements: high-rate SBR treatment, anaerobic sludge digestion and anammox. The following is a summary of key research outcomes from this project:

- The high-rate SBR activated sludge process can effectively treat abattoir wastewater, achieving around 80% COD and P removal and 55% N removal, while generating an effluent suitable for irrigation or sewer discharge. The high-rate process is compact, with an HRT of 0.5–1 day, and SRT of 2–2.5 days.

- Bio-P removal was achieved in the high-rate SBR, more efficiently at 2–2.5 days SRT compared with longer SRTs of 3–4 days. Bacterial cells of the family *Comamonadaceae*, arranged in tetrads, contained positive DAPI-stained polyP inclusions, indicating that *Comamonadaceae* is a strong candidate as the PAOs responsible for the high-rate Bio-P removal process demonstrated for the first time in this project.

- The short sludge-age (2–4 days) activated sludge produced from the high-rate SBR process can be successfully digested through thermophilic, mesophilic or TPAD processes, with 60% degradability obtained with four days, rising to over 80% at two days SRT. Thermophilic and TPAD processes enhanced the sludge degradation rate, but not the degradation extent, compared to the mesophilic process.

- The continuous anaerobic digestion study (using two-day SRT sludge) demonstrated that a VS destruction of 60–70% could be achieved in the thermophilic digester (55°C and 8–10 days HRT), which was considerably higher than in the mesophilic digester (35°C and 10 days HRT). Given the higher treatment performance and better pathogen removal, a thermophilic process is considered the most attractive solution.

- The sludge dewatering liquor from the thermophilic anaerobic digester can be effectively treated via anammox to largely remove the residual N, indicating a major potential for successful full-scale application of this highly efficient and effective N-removal process.

- The cost–benefit analysis showed that this integrated high-rate system provides a very compact and energy-positive treatment option for wastewater resulting from meat processing. Using smaller reactors means there is substantial reduction in the amount of space required. There is a considerable net energy output due to the reduced aerobic oxidation, and a concurrent potential for increased methane production due to efficient sludge digestion. Remarkably, a net operating benefit is expected to be achievable due to the (renewable) power generation potential of this process.

8.0 Recommendations

Based on the results in this project, the following aspects of the research are recommended for further evaluation, with the aim of implementing this new wastewater treatment system in the Australian red meat processing industry.

- The flow and composition of wastewater from red meat processing can vary considerably across sites, and over time even within one site. This could result in overloading the high-rate SBR activated sludge process during daytime and/or the system starving at night. Periodical overloading of fats and solids can cause a sudden increase of aeration demand, leading to the DO concentration in the process being lower than the target value, which negatively affects the effluent quality. It is important to develop an appropriate operational control and optimisation scheme for the process, to ensure the consistently good performance of the system. Using a buffering process (such as a buffering tank) prior to the SBR should also be considered as a means of equalising and balancing fluctuating wastewater flows and concentrations.

- Anaerobic digestion is used in this project to convert carbon captured in the activated sludge to methane, but could also be used to treat other solids streams produced during red meat processing (including paunch and cattle washing) and to offer additional benefits. Co-digestion options should be considered and evaluated to maximise the potential methane yield and better use the infrastructure investment in these systems.

- Phosphorus organic compounds contained in the activated sludge were released in the form of PO_4^{3-}-P during anaerobic digestion (up to 230 mg P L^{-1}), suggesting a great potential of P recovery using struvite crystallisation. The feasibility and costs of this process should be further evaluated.

- Anammox was tested as a side-stream treatment process for removing the residual N in the sludge dewatering liquor, and should be expanded to be used in the mainstream treatment to eliminate NH_4^+-N remaining in the SBR effluent. Further investigation is recommended to assess the impact of non-degradable COD on anammox activity, to determine efficient ways

of removing N, and to predict the total nitrogen and ammonium concentrations in the SBR effluent compared to discharge limits for the Australian red meat processing industry.

9.0 References

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Appendix I

Anaerobic Biodegradability of Sludge Generated From High-Rate SBR

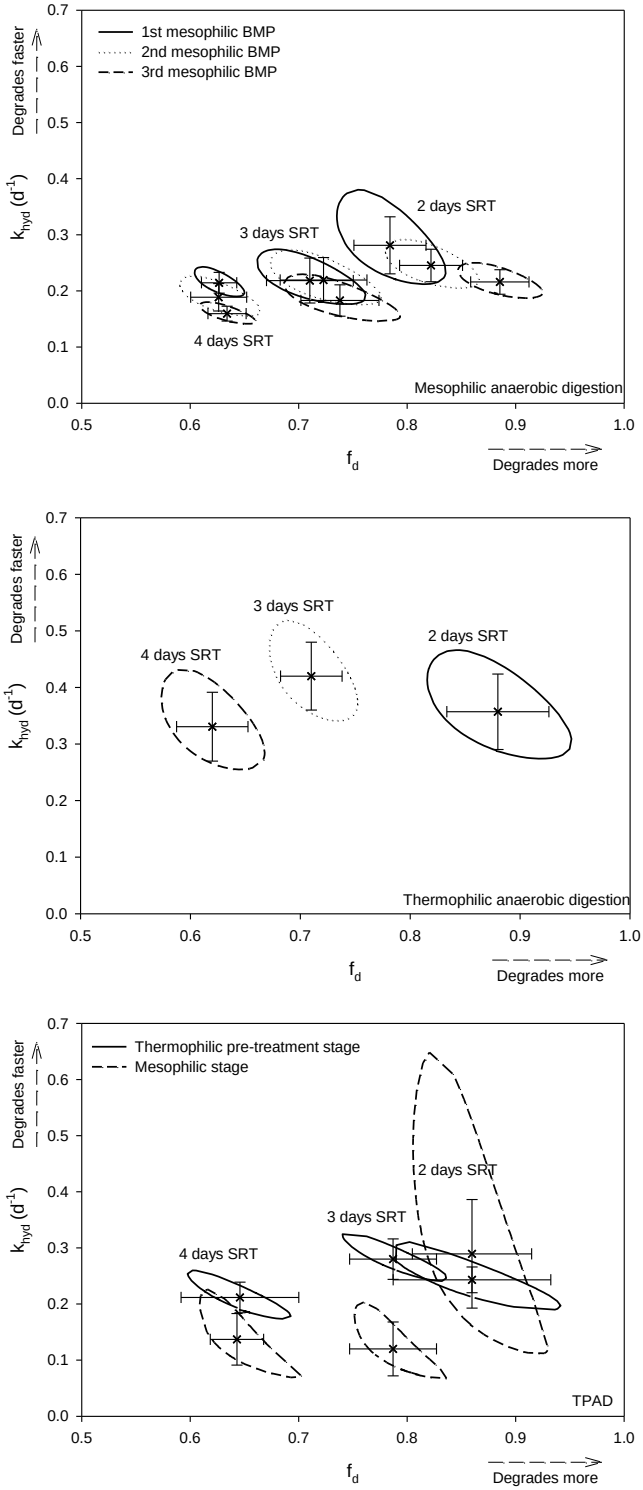

Figure 19: Confidence regions of k_{hyd} and f_d for mesophilic anaerobic digestion (37°C), thermophilic anaerobic digestion (55°C) and TPAD (55–37°C), treating the waste activated sludge with two-day SRT, three-day SRT and four-day SRT generated from the high-rate aerobic stage. The digestion test for each sludge was repeated twice under mesophilic anaerobic digestion.

Appendix II

Anaerobic Digestion Performance of Continuous Digesters Treating Sludge Generated From High-Rate SBR

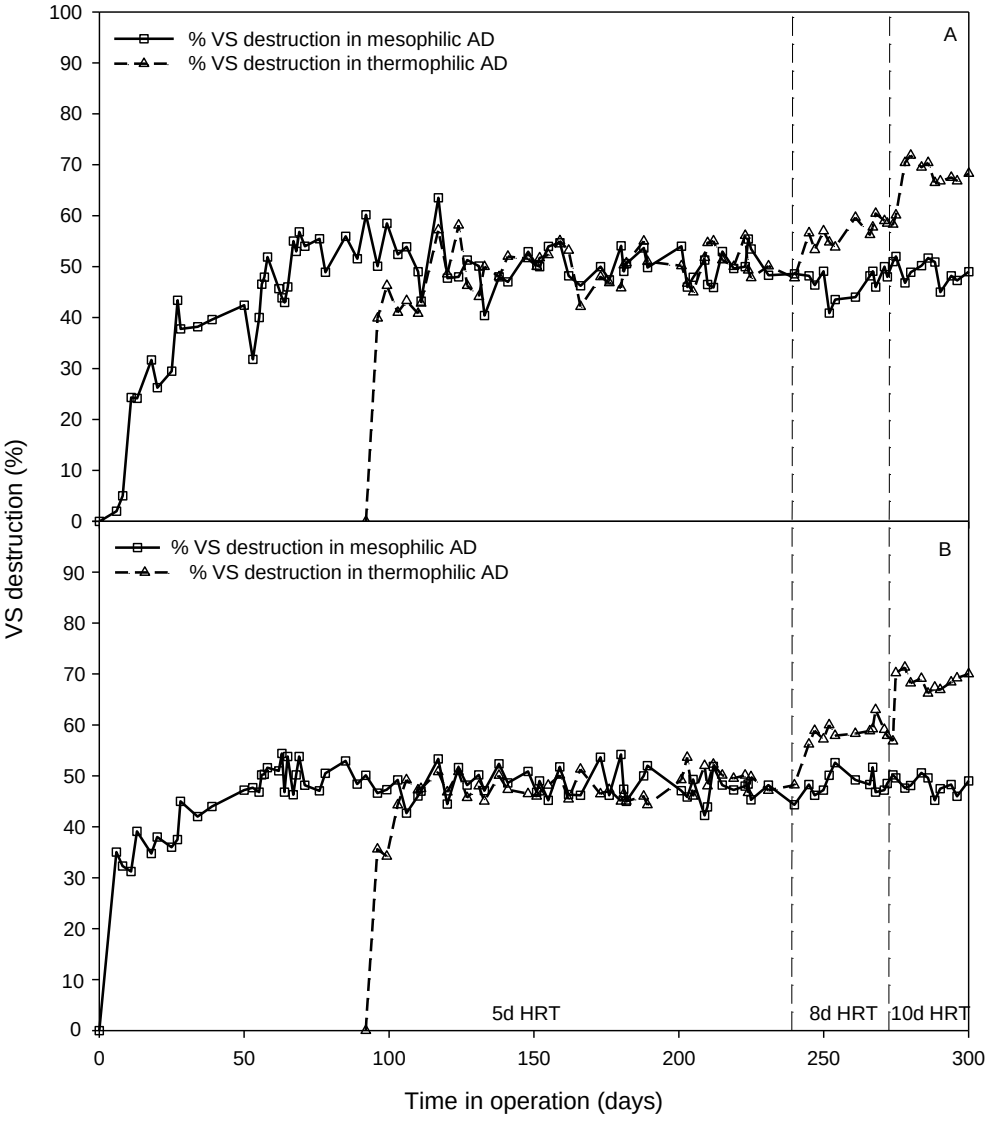

Figure 20: VS destruction calculated by mass balance equation (A) and Van Kleeck equation (B) during each period in the thermophilic and mesophilic anaerobic digesters. The % VS destruction is based on the activated sludge feed characteristics.