AUSTRALIAN MEAT PROCESSOR CORPORATION

Purple phototrophic bacteria for resource recovery from red meat processing wastewater

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1.0 Executive Summary

Purple phototrophic bacteria (PPB) are an emerging technology that enables removal of organics, nitrogen and phosphorous from wastewater streams in a single treatment step by concentrating within biomass. The batch assessment of the treatability showed efficient COD, nitrogen and phosphorous removal while removal was assimilative with partitioning from the liquid to the solid phase, enabling the recovery as protein-rich biomass. Alternatively, the biomass can be anaerobically digested but the economic feasibility would be reduced. The major findings of the batch tests are:

- Combined wastewater entering primary treatment (such as dissolved air floatation) can be treated using PPB achieving 92% NH_4-N removal and 25% PO_4-P removal whereby TN and TP are persevered in the biomass. The portion of COD converted to protein biomass could not be determined due to the particulate nature of COD in the feed. Improved performance may be achieved through optimisation.
- When combined wastewater was pre-filtered to remove particulates, PPB treatment resulted in 74% SCOD, 64% NH_4-N and 73% PO_4-P being removed from the wastewater and converted to microbial protein. Improved performance may be achieved through optimisation.
- Time series data showing nutrient removal during treatment indicates process retention time of 2 days or less can be achieved through optimisation.
- The PPB biomass product had a crude protein content above 60%, confirming potential to generate a value-add product.
- Using a photo anaerobic membrane bioreactor (PAnMBR), a PPB process would generate microbial protein product with higher crude protein and energy content compared to existing byproducts (Meat and Bone Meal) or competitor products (Cotton Seed Meal). This could generate a potential product stream with high value ($400-600 t^{-1}). This strategy is strongly recommended for further development.
- Anaerobic digestion of PPB biomass for energy recovery will results in lower value recovery and will require secondary treatment of the digestate, further reducing the value proposition. This strategy is not recommended for further research.
- PPB biomass can be anaerobically digested to generate renewable biogas energy. Methane yields are higher than existing sludge treatment processes, however revenues are lower and this option creates a requirement for secondary effluent treatment.
- The capital costs of the PAnMBR process without dedicated solids treatment is approximately 40% more expensive compared to developing configurations such as CAL+ anammox and CAL + Algae Pond. However, capital costs are lower compared to AnMBR + anammox, High rate SBR + AD + anammox, CAL+BNR or CAL+BNR (WAS recycle).
- PPB processes are a developing technology and lack of references on red meat wastewater means there are several key research questions that must be addressed, particularly around performance of PPB in systems with solid COD and fat.

The outcomes of this project demonstrate PPB technology for red meat processing wastewater treatment is a new and potentially disruptive technology for generation of value-add products that could substantially change the economics of wastewater treatment. Preliminary results demonstrate process feasibility and continued research to develop a continuous process is recommended. Future work should incorporate more detailed characterisation of the PPB biomass and the application as high value, protein rich feed or feed additive.

Abbreviations

AAR	Anaerobic Ammonium Removal
AD	Anaerobic Digestion
AL	Anaerobic Lagoon
Anammox	Anaerobic Ammonium Oxidation
AnMBR	Anaerobic Membrane Bioreactor
ATP	Adenosine triphosphate – transports chemical energy within living cells
BChl	Bacteriochlorins
BNR	Biological Nitrogen Removal
C	Carbon
CAL	Covered Anaerobic Lagoon
Chl	Chlorins
COD	Chemical Oxygen Demand
DAF	Dissolved Air Flotation (tank)
DO	Dissolved Oxygen
FOG	Fat, Oils and Grease
HRAP	High Rate Algal Ponds
HRT	Hydraulic Residence Time
IR	Infra-red
MBM	Meat and Bone Meal
N	Nitrogen
NH_4-N	Ammonium nitrogen
NO_2-N	Nitrite Nitrogen
NO_3-N	Nitrate Nitrogen
P	Phosphorus
PAnMBR	Phot Anaerobic Membrane Bioreactor
PO_4-P	Phosphate Phosphorus
PPB	Purple Phototrophic Bacteria
SBR	Sequencing Batch Reactor
SRT	Sludge Retention Time
TKN	Total Kjehldahl Nitrogen
TP	Total Phosphorus
TS	Total Solids
TSS	Total Suspended Solids
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VS	Volatile Solids

WAS	Waste Activated Sludge
WSP	Waste Stabilisation Ponds

2.0 Introduction

Purple phototrophic bacteria (PPB) for wastewater treatment are a novel concept that allows near complete carbon, nitrogen and phosphorous removal in a single step process along with the ability to produce a homogenous biomass product as a single cell protein product. PPB partition soluble organics and nutrients into biomass and removal occurs simply by microbial growth, rather than reaction and dissipation as in conventional aerobic and anaerobic processes. At the same time, PPBs use organics and nutrients as building blocks for protein synthesis and more than 65% of the biomass can be protein. In fact, PPB technology can produce a concentrated protein product while simultaneously treating the wastewater. It therefore fully preserves these compounds in an organic solid concentrate stream suitable for recovery and export. PPB have been applied to domestic wastewater treatment (Hülsen et al. 2014, Hülsen et al. 2016a), but have not previously been applied to meat processing wastewater in a continuous or commercial process.

This project focussed on assessing the initial feasibility of purple phototrophic bacteria for treatment of meat processing wastewater and simultaneous value-adding to waste. Feasibility is assessed through laboratory batch tests designed for evaluate wastewater treatment (organic, nitrogen and phosphorous) efficiency but also biomass product characteristics (protein, fat and carbohydrate contents) as well as the biological methane production from biomass generated during the wastewater treatment. The findings are used to set-up a cost-benefit-analysis for PPB as a wastewater treatment process but also considers end use of the PPB product through either direct use or anaerobic digestion.

The project builds on current industry research and development in this area, including:

- A.ENV.0164 Feasibility study into the application of anaerobic ammonium removal technology for wastewater treatment at red meat processing facilities
- A.ENV.0132/0150 High Rate aerobic treatment with AD and anammox
- A.ENV.0133/0149 Integrated agro industrial wastewater treatment and nutrient recovery
- A.ENV.0154 Nutrient recovery from paunch and DAF sludge (struvite)
- A.ENV.0151 NGERS and Wastewater Management – mapping waste streams and quantifying the impacts.
- A.ENV.0162 Review and evaluation of the application of anaerobic ammonium removal technology for wastewater treatment
- 2016/1023 Milestone 2- Meat processing wastewater treatment with purple phototrophic bacteria-a review

2.1 Project Objectives

Project objective as described in the project contract are:

a) Determine if PPB can be selectively enriched from slaughterhouse wastewater using only infra-red (IR) light as driver;
b) Determine what effluent nitrogen, phosphorous and organics (COD) levels can be achieved;
c) Determine the resulting microbial material, and the digestibility of the product;
d) Determine the cost-benefit of the process.

Project Scope

The project was completed in two stages.

Stage 1: Desktop analysis and basic feasibility

- Conduct complete literature review, including competitive analysis against photosynthesis (algae and cyanobacteria), and chemosynthesis (using chemical energy), as well as ex-ante cost benefit analysis, value proposition, and SWOT analysis of the technology.
- This represented a hold point with the following criteria:
    - Capital cost estimates are order of magnitude comparable with existing platforms (lagoons and anaerobic membrane bioreactor processes).
    - Return on investment for a range of product utilisation options comparable with existing processes (i.e., >10% ROI).
    - Market analysis to identify clearly value of potential products.

Stage 2: Technical feasibility through batch testing

- Batch tests were done in triplicate 160 mL serum flasks to enable:
    - Identification of the degree and rate at which PPB are enriched from slaughterhouse wastewater at different light levels.
    - Use of enriched biomass to assess the level to which PPB can be used to remove carbon, nitrogen, and phosphorous from red meat processing wastewater.
    - Further use of enriched biomass to determine affinity for organics in slaughterhouse wastewater to enable process modelling using existing UQ expertise in metabolic modelling.
    - Testing of basic degradability and digestibility according to animal metabolic proxies, and anaerobic batch tests.
- Stage 2 was due to complete approximately 10-12 months after the project start, with the following major goals:
    - PPB successfully enriched in batch (dominant functional microbe).
    - The process can achieve removal from soluble phase of carbon-nitrogen-phosphorous to levels competitive with next generation treatment technologies (COD<300 mg/L, N<10mgN/L).

Basic digestibility parameters of PPB are expected to be compatible with product utilisation in a range of applications.

3.0 Literature Review

This section presents an overview of wastewater production in the Australian red meat processing industry and outlines purple phototrophic bacteria (PPB) as a potential treatment option. A more detailed review of PPB literature is presented in Appendix B.

Production of Wastewater at Red Meat Processing Facilities

Australian red meat processing facilities generate large volumes of wastewater rich in organic contaminants and nutrients (Johns 1995, Liu and Haynes 2011). The wastewater is relatively concentrated with total organics in the order of 10,000 mg L^{-1} as COD, with high nitrogen and phosphorous levels. While potentially expensive, the removal of these contaminants is necessary in order to comply with water discharge regulations. These contaminants also make red meat processing facilities strong candidates for advanced treatment processes aimed at removal and/or subsequent recovery of energy, nutrient and water resources.

Processes such as covered anaerobic lagoons (CAL) and high-rate anaerobic membrane processes (AnMBR) generate revenue on the basis of energy recovery (payback 2-5 years) but leave residual nitrogen (200-400 mgN L^{-1}) and phosphorous (up to 50 mgP L^{-1}). The wastewater can be irrigated, but this generally requires very large land footprints; or discharged to sewer, but this can result in excessive trade waste charges (\$0.95 kL^{-1}, \$0.93 $kgBOD^{-1}$, \$1.80-2.10 kgN^{-1} and \$1.70-4.20 kgP^{-1}; QUU 2014/15 trade waste charges). In general:

- Existing treatment practices such as crusted or covered lagoons remove organics, but do not reduce N or P.
- Emerging nutrient recovery technologies, such as struvite precipitation are effective for P removal, but not suitable as a stand-alone technology for or N recovery.
- Emerging processes such as anaerobic ammonium removal (i.e. anammox) allow economic removal of N, and are nearer to market, but do not offer the possibility for nitrogen or value-add product recovery.

These existing and developing wastewater technologies target specific contaminants in the wastewater and are not suitable as stand-alone technologies. The novel PPB process is a possible alternative, able to remove COD, N and P in one step.

Waste and wastewater originates from several major process operations at a slaughterhouse including cattle preparation, cattle slaughter, recovery of by-products and reprocessing of by-products (Liu and Haynes 2011). Generally, waste streams from different processing areas are transported separately within the site then combined for bulk treatment (e.g. in an anaerobic lagoon). The structure of waste and wastewater handling processes varies between sites; however a recent investigation of 6 Australian meat processing facilities identified common trends (Jensen et al. 2014a). A general structure of wastewater handling practices is presented in Figure 1. Combined slaughterhouse wastewater is composed of a mixture of grease, fat, protein, blood, intestinal content, manure and cleaning products (Johns 1995). It contains high concentrations of organic matter (represented by chemical oxygen demand, COD); oil and grease (FOG); nitrogen (N); phosphorus (P) and other trace metals.

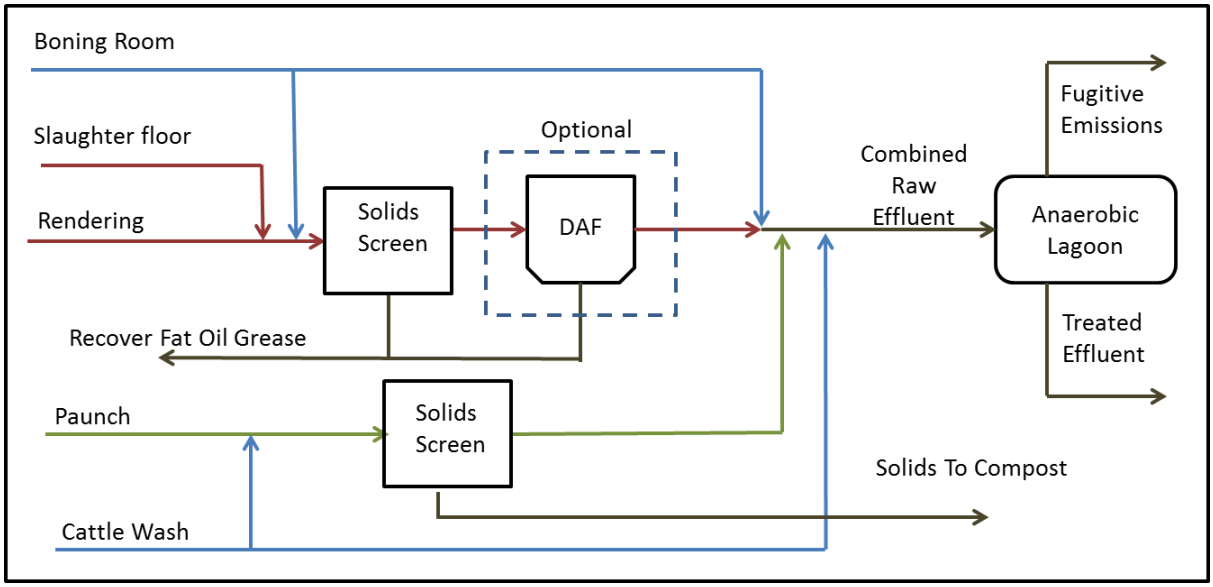

Figure 1: Major wastewater sources and generalised structure of waste and wastewater handling practices at Australian red meat processing sites (Jensen et al. 2014b)

The composition of combined wastewater at these Australian red meat processing facilities is shown in Table 1, while the compositions of slaughterhouse wastewater as reported in international studies are shown in Table 2. The comparison shows that wastewater from Australian slaughterhouses is concentrated by international standards, both in regards to organic contaminants (COD) and nutrient (N and P).

Table 1: Characteristics of Australian slaughterhouse wastewater after primary treatment/solids removal (A.ENV.0131 and A.ENV.0151).

	Volume $m^3 d^{-1}$	TCOD mg L^{-1}	sCOD mg L^{-1}	FOG mg L^{-1}	N mg L^{-1}	P mg L^{-1}	*TCOD:TN:TP ratio
Literature Concentration	-	2,000-10,000	-	100-600	100-600	10-100	100:6.0:1.0
Site A	2420	12,893	1,724	2,332	245	53	100:1.9:0.4
Site B	3150	9,587	1,970	1,300	232	50	100:2.4:0.5
Site C	2110	10,800	890	3,350	260	30	100:2.4:0.3
Site D	2150	12,460	2,220	3,300	438	56	100:3.4:0.4
Site E	1600	12,200	1,247	2,380	292	47	100:2.4:0.4
Site F	167	7,170	1,257	2,258	182	27	100:2.5:0.4

*based on maximum values

Table 2: Characteristics of slaughterhouse wastewater after primary treatment/solids removal (Lemaire 2007).

Reference	Country	TCOD mg L^{-1}	SCOD mg L^{-1}	FOG mg L^{-1}	TKN mgN L^{-1}	TP mgP L^{-1}	*TCOD:TKN:TP ratio
Borja et al. (Borja et al. 1994)	Spain	5,100	-	-	310	30	100:6.1:0.6
Caixeta et al. (Caixeta et al. 2002)	Brazil	2,000-6,200	-	40-600	-	15-40	100:XX:0.7
Li et al. (Li et al. 1986)	China	628-1,437	-	97-452	44-126	10-16	100:8.6:1.1
Manjunath et al. (Manjunath et al. 2000)	India	1,100-7,250	-	125-400	90-150	8-15	100:5.5:0.2
Martinez et al. (Martinez et al. 1995)	Spain	6,700	2,400	1,200	268	17	100:4:0.3
Nunez and Martinez (Núñez and Martínez 1999)	Spain	1,440-4,200	720-2,100	45-280	-		
Russell et al. (Russell et al. 1993)	NZ	1,900	-	-	115	15	100:6.1:0.8
Sachon (Sachon 1986)	France	5,133	-	897	248	22	100:4.9:0.4
Sayed et al. (Sayed et al. 1987)	Holland	1,500-2,200	-	-	120-180	12-20	100:8.2:0.9
Sayed et al. (Sayed and De Zeeuw 1988)	Holland	1,925-11,118	780-10,090	-	110-240	13-22	100:2.2:
Stebor et al. (Stebor et al. 1990)	US	4,200-8,500	1,100-1,600	100-200	114-148	20-30	100:1.7:0.4
Thayalakumaran et al. (Thayalakumaran et al. 2003)	NZ	490-2,050	400-1,010	250-990	105-170	25-47	100:8.3:2.3

*based on maximum values

3.1 Current Wastewater Treatment Practices at Red Meat Processing Facilities

Generally, waste streams from different processing areas are transported separately within the site then combined for bulk treatment (e.g. in an anaerobic lagoon). The structure of waste and wastewater handling processes varies between sites but the general processes in Australia include dissolved air flotation (DAF) as a pre-treatment to remove fat, oil and grease (FOG) and total suspended solids (TSS).

The DAF effluent is fed to an anaerobic treatment step. Anaerobic lagoons with hydraulic retention times (HRT) ranging between 7 and 14 days (Lemaire et al. 2009) are commonly used and are effective at removing organic material (COD); however lagoon based processes also have major disadvantages including large footprints, poor gas capture, poor odour control, limited ability to capture nutrients and expensive de-sludging operations. Even in warmer climates, there is an emerging and strong case for reactor based technologies with focus on anaerobic biogas generation. In the anaerobic step, organics will be converted to biogas and the organic bound nitrogen will be released as ammonium. Reliable biological COD and nitrogen removal systems have been successfully developed and applied for abattoir wastewater treatment using continuous activated sludge systems (Beccari et al. 1984, Frose and Kayser 1985, Willers et al. 1993). However, removal of nitrogen through reactive biological processes requires energy input in aeration and carbon chemical addition. Novel removal technology such as the anammox process offer economic nitrogen removal with no need of external COD addition, but reactively removes ammonium as nitrogen gas. PPB is another emerging option to replace these existing (conventional) technologies for COD, N and P removal, with reductions in cost, energy consumption, footprint and elimination of chemical addition.

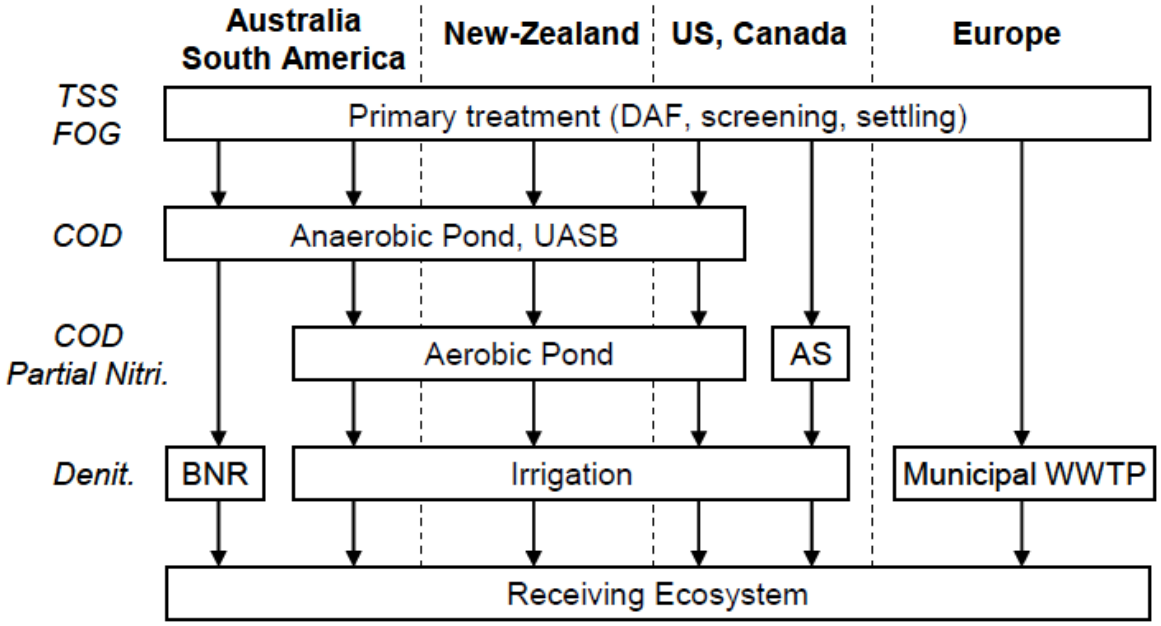

Figure 2: Principal wastewater treatment set-up of the meat industry (Lemaire 2007). Note: At some smaller Australian plants, primary treatment may be bypassed and/or raw effluent may be used for irrigation or land application.

3.1.1 Technologies for Removal of Organics

A brief summary of technologies for removal of organic contaminants and operational considerations for application to meat processing wastewater is shown in Table 3.

Table 3: Summary of anaerobic digestion technologies

Technology	Principle	Advantages	Disadvantages	Loading rate ($kgCOD.m^{-3}d^{-1}$)	COD removal efficiency
Crusted Anaerobic Lagoon	Large retention time, partially mixed vessel.	Very low capital cost	Very high footprint. Must be desludged. No methane capture/high carbon liability. Can produce odours. Very limited controllability	0.1	70-80%
Covered Anaerobic Lagoon	Large retention time, partially mixed vessel.	Low capital cost	Very high footprint. Must be desludged. Methane capture average. Can produce odours. Very limited controllability	0.1	70-80%
High Rate Anaerobic (Granular)	Mainly liquid wastewater flows upwards through a granular bed.	Low footprint, low capital cost, very stable, produces good effluent.	Intolerant to solids. Intolerant to fats.	10 (UASB) 20 (EGSB/IC)	80-90%
Anaerobic Membrane Bioreactors	Mainly liquid wastewater flows through a membrane that retains solids.	Low footprint, low capital cost, very stable, produces good effluent.	Moderate to high operating costs related to membrane.	3-6	>95%
Mixed Liquor digesters	Dilution to 3-6%, and continuous feed in mixed tank. Retention of 20 days. Used across many industries	Established tech Easy to control Continuous gas production	Poor volumetric loading rate Expensive tanks Need dilution liquid Liquid (not solid) residue	1-3	60-80%
Aerobic lagoons	Large retention times partially mixed vessel	Low capital costs Less odour problems	Very high footprint Must be de-sludged no methane production series of lagoons necessary	0.1 -0.3	80-90%
Conventional Activated sludge	Medium retention times Biomass settling with clarifiers and sludge recycling	Medium footprint Low capital costs Low operating costs produces good effluent	High sludge production Produces sludge side-stream No methane production	0.2 – 0.6	80-90%
PPB	IR light is used to drive uptake of COD, N and P into biomass	Simultaneous removal in one step, Low N and P	New technology, research needed Potential for high capital costs	1.0-10	Up to 95%

4.0 Technologies for Removal of Nutrients

A brief summary of technologies for removal of nutrient contaminants and operational considerations for application to meat processing wastewater is shown in Table 4.

Table 4: A comparison of the process features of different nutrient removal technologies.

Technology	Volumetric loading rates ($kg.m^{-3}d^{-1}$)	TN removal (%)	Energy demand ($kWh\ kgN_{removed}^{-1}$)	Chemical Costs ($\$\ kgN_{removed}^{-1}$)	Sludge Production ($kgTSS\ kgCOD^{-1}.d^{-1}$)	Start-up (months)	Other process issues
Anammox	0.7-2.0	70-90% TN	1.0-1.8	-	~0.05	Up to 4 months	Poor tolerance to FOG
Nitrification/ Denitrification	0.1-0.3	Over 95% TN	4.6	-	0.2-0.4	Less than 1 month with inoculum	Sludge disposal costs or side-stream treatment train needed
Stripping	TBC	70-90%	25 including chemicals [22]	Included in energy demand	N/A	Less than 1 month	Only feasible at high NH_4-N >3000mg L^{-1}
Wetlands	TBC	Up to 70% TN	N/A	-	N/A	>12month	Very large footprint, limited removal efficiency
Crystallization	3-May	TP removal above 90%, but TN removal <20%	5.8 including chemicals [22]	Included in energy demand	N/A	Less than 1 month	Low value fertiliser
PPB	Based on COD	Over 95% TN	1-2kWh For COD, N and P removal	-	0.8-0.95	Less than 3 month without inoculum	New technology, research needed

5.0 Introduction to Purple Phototrophic Bacteria

Purple phototrophic bacteria (PPB) are commonly distributed in the natural environment in soil, fresh water,- marine environments,- and wastewater and can be readily isolated from these sources (Zhang et al. 2003).

PPB generate chemical energy from light rather than from other chemicals (Basak and Das 2007). This is a prerequisite for high biomass yields and avoids gaseous emissions due to product formation. The capability to generate energy from light is related to the presence of either chlorins (Chl) or bacteriochlorins (BChl) which are photosynthetic pigments that occur in various phototrophic organisms. Different pigments allow the organism to utilise different light spectrums. Table 4 gives an overview of BChls and Chl and the absorption maxima. Furthermore, the carotenoids give the PBB culture specific colour, ranging from yellow, orange to red (Blankenship et al. 1995). Anoxygenic photosynthesis, where light energy is captured and converted to ATP without the production of oxygen, is a key mechanism in the proposed PPB process and has been extensively studied and reviewed (Blankenship et al. 1995). Further readings about the biochemistry and molecular structures of the light harvesting complexes (LHC) can be found elsewhere (McEwan 1994, Madigan and Martinko 2006).

Table 5: Absorption maxima of different chlorins.

*BChl and Chl	Wavelength or wavelength range of absorption maxima (nm)
BChl *a*	375, 590, 805, 830-911
BChl *b*	400, 605, 835-850, 986-1035
Chl *c*	457-460, 745-755
Chl *d*	450, 715-745
Chl *e*	460-462, 710-725
BChl *g*	375, 419, 575, 788

Adapted from (Overmann and Garcia-Pichel 1998).
*BChl *a*, *b*, and *g* are bacteriochlorins, Chl *c*, *d* and *e* are chlorins.

Importantly, PPB contain BChl a and/or BChl b, these pigments enable them to absorb light in the near infra-red (NIR) and this capability is not shared by other phototrophs such as algae or cyanobacteria (Bertling et al. 2006). Therefore, IR light provides PPB with a distinct competitive advantage and can be used to select for phototroph communities of PPB. The capability of PPBs to utilise IR is also a distinct operational advantage as IR light from light emitting diodes (LEDs) can save up to 70% of the power requirements compared to white light (Bertling et al. 2006), needed for algae growth. Finally, since phototrophs utilise organics for growth rather than CO_2 (photosynthetic), the light input per gram biomass is far less.

6.0 Application of PPB for Wastewater Treatment

PPB have high potential in the treatment of wastewater due to removal of COD (Azad et al. 2001) but also removal of phosphorous through polyphosphate (polyP) formation (Hiraishi et al. 1991), removal of NO_3-N by denitrification (Kim et al. 1999, Satoh et al. 1976), removal of NH_4-N by assimilation (Takabatake et al. 2004) and odour reduction due to H_2S assimilation and oxidation (Nagadomi et al. 2000). At the same time, valuable products can be produced such as; polyhydroxybutyrate (PHB) (Khatipov et al. 1998) bio-hydrogen (Wu et al. 2012), oxycarotenoids (Ponsano et al. 2004) and the PPB biomass itself with potential applications as organic fertiliser (Xu 2001) or animal feed (Kobayashi and Tchan 1973).

Several PPB strains have been isolated from various sources for wastewater treatment. Table 6 shows a variety of studies treating wastewaters with different species of PPB. These studies show the potential for PPB in wastewater treatment. However, many of these studies were not based on realistic real word conditions and utilised axenic cultures grown in predefined media as inoculum (i.e. cultures with only the desired organisms). Most of the experiments were conducted with synthetic wastewater or sterilized influent which avoids competition with other organisms and does not represent a true industry application.

However, PPB have been applied to non-sterile wastewater, achieving satisfying COD removal from sardine processing wastewater by *Rhodovulum sulfidophilum* (Azad et al. 2001, Azad et al. 2004). Kantachote et al. (2010) used *Rhodopseudomonas palustris* to remove COD and H_2S from a mix of raw rubber sheet wastewater and fermented plant extracts. COD removal from tuna condensate and a mix of tuna condensate and shrimp-blanching water by *Rhodocyclus gelatinosus* grown in G5 medium was reported by Prasertsan et al. (1993). These wastewaters contain high concentrations of COD (7.0 up to 60 g L^{-1}) but low N and P.

PPB have been applied successfully for domestic wastewater in batch tests (Hülsen et al. 2014) as well as in continuous lab-scale photo anaerobic membrane bioreactors at ambient (Hülsen et al. 2015 in submission) and cold temperatures (Hülsen et al. 2015). PPB were able to remove organics, nitrogen and phosphorous simultaneously, in one step to below discharge limits (COD <100 mg L^{-1}, TN <10mg L^{-1} and TP <1mg L^{-1}). For every 100 g of SCOD, 8 g of NH_4-N and 1.3 g of PO_4-P are assimilated, resulting in a SCOD:NH_4-N:PO_4-P substrate ratio of 100:8:1.3. This concept was proposed as a new platform for wastewater treatment of the future, including the recovery of heat energy and fertilisers due to non-destructive assimilative treatment (Batstone et al. 2014). However, publications describing the full-scale application of PBB to treat wastewater are currently limited and this creates some uncertainty.

Table 6: Summary of wastewater treated with PPB.

Wastewater	Pre-treatment	$COD_{removed}$	PO_4-P $_{removed}$	NH_4-$N_{removed}$	HRT	Light	PPB	Ref
		%	%	%	d	Lux	-	-
Noodle Processing WW	-	90			6-10		Rps. Palustis and Rba. Blasticus	(Chiemchaisri et al. 2008)
Tilapia fish processing WW	filtered, pasteurized	43		22.5 (TN)	3-7	1,400 ± 200	R. gelatinosus	(de Lima et al. 2011)
Sardine processing WW	-	71			5	2500	R. sulfidophilum	(Azad et al. 2001)
Sardine processing WW	settling	77			5	2500	R. sulfidophilum	(Azad et al. 2001)
Latex processing WW	autoclaving	57			1.7	3000	R. gelatinosus	(Choorit et al. 2002)
Swine WW	autoclaving	90 (diluted), 50 (undiluted)	58		6	4000	Rps. palustris	(Kim et al. 2004)
Tuna condensate	1:10 dilution	78			5	3000	R. gelatinosus	(Prasertsan et al. 1993)
Tuna condensate and shrimp blanching water	-	86			5	3000	R. gelatinosus	(Prasertsan et al. 1993)
Food processing WW	-	518(MBR), 48 (SBR)			10	IR	mix	(Chitapornpan et al. 2012)
Olive mill WW	dilution	33 CL, 31 (dark /light)				200 W m^{-2}	R. sphaeroides	(Eroglu et al. 2010)
Poultry slaughterhouse WW	filtered, pasteurized	91			10	4000 ± 500	R. gelatinosus	(Ponsano et al. 2008)
Pharmaceutical WW	add $(NH_4)_2SO_4$ and yeast extract	80			5	6000	R. sphaeroides	(Madukasi et al. 2010)
Sardine processing WW	-	85			5	2500	R. sulfidophilum	(Azad et al. 2004)
Synthetic sewage WW	-	89	77	99 (NO3-N)	2	-	R. sphaeroides Rps. palustris	(Nagadomi et al. 2000)
Latex rubber sheet WW	add $(NH_4)_2SO_4$ and nicotinic acid, centrifuged	90			4	3000	Rps. blastica	(Kantachote et al. 2005)
Latex rubber sheet WW	filtering	80			3	-	R. palustris	(Kantachote et al. 2010)
Sulfate containing food industry WW	-	90			3-10	45 W m^{-2}	Mix	(Chiemchaisri et al. 2007)

7.0 Experimental Methods

Source of Purple Phototroph Bacteria

PPBs were enriched from domestic wastewater using a previously described methodology to provide inoculum for the batch tests (Hülsen et al. 2014). Following the enrichment, the PPB cultures were incubated under anaerobic conditions and exposed to light at 30±1°C in 10 L Schott bottles. Each bottle was continuously stirred at 200 rpm (RCT basic, Kika Labortechnik) and illuminated with a 150W fluorescence lamp (Nelson Clamp Flood Light). The illumination intensity was 50 $W.m^{-2}$ on the outside of the bottle and each bottle was covered with UV-VIS absorbing foil (ND 1.2 299, Transformation Tubes). The bottles were fed weekly with 90% fresh domestic wastewater and 500 mg acetic acid as additional substrate (HAc). After HAc addition, the pH was adjusted to 6.7 using 6M sodium hydroxide.

For each batch test, the PPB culture was concentrated to around 10 g L^{-1} by centrifuging at 3270 x *g* for 12 minutes at 20°C (Allegra™ X-12 centrifuge) for form a PPB pellet. The pellet was re-suspended in small volumes of supernatant (liquid fraction after centrifuging). The concentrated PPB inoculum was analysed in terms of; TCOD, SCOD, TSS/VSS, TKN, TP, elemental analysis, NH_4-N, NO_3-N, NO_2-N and PO_4-P, VFA and microbial composition (16s amplicon sequencing).

Ormerod Medium

Ormerod medium was used in separate control experiments to measure the baseline activity, yield, nitrogen and phosphorous uptake of the PPB inoculum – these experiments did not contain wastewater. The medium was a modified version of that described by Ormreod at al. and contained Acetic acid, 0.5 g.; K_2HPO_4, 900 mg.;KH_2PO_4, 600 mg.; $MgSO_4$ x $7H_2O$, 266 mg.; $CaCl_2$ x $2H_2O$, 75 mg.; $FeSO_4$ x $7H_2O$, 11.8 mg; ethylenediaminetetraacetic acid, 20 mg.; yeast extract, 15 µg.; trace element solution (per 100 ml of deionized water: H_2BO_3, 280 mg; $MnSO_4$ x $4H_2O$, 210 mg; Na_2MoO_4 x $2H_2O$, 75 mg.; $ZnSO_4$ x $7H_2O$, 24 mg.; $Cu(NO_3)_2$ x $3H_2O$, 4 mg), 1 ml; and NH_4Cl, 0.5 g. The pH of the medium was adjusted to 6.8 with NaOH (Ormerod et al. 1961).

Batch Test Set-up

For the filtered and raw wastewater tests, 9 serum flasks (160 mL) were used to measure 3 sets in triplicate. PPBs were illuminated with two fluorescence lamps with UV-VIS absorbing foil under anaerobic conditions and continuously shaken at 100 rpm at 30°C (Orbital shaker, MaxQ4000, Thermo Scientific, Australia). Table 7 shows the incubation conditions for each triplicate. Additionally, non-filtered DAF influent was illuminated and incubated without PPB inoculum to test the development of a phototrophic consortium directly from the wastewater. Each wastewater test took 3 to 4 days and TCOD, SCOD, NH_4-N, NO_3-N, NO_2-N and PO_4-P were measured 3 times the first day, 2 times the second day and once a day afterwards. The illumination intensity was 10 Wm^{-2} for each PPB batch test.

Table 7: Test conditions (all tests done in triplicate)

Medium and biomass type	Illumination
Wastewater + PPB	+
Ormerod medium + PPB	+
Wastewater control	-
Wastewater control*	+

*only for non-filtered DAF influent.

8.0 Chemical Analysis

Chemical oxygen demand (COD) was determined according to Standard Methods 5220D with potassium dichromate in sulfuric acid as the key reaction chemicals in colorimetric tests using Merck Spectroquant® COD cell tests (114560, 114540, 114541 and 114555 AD).

Volatile fatty acids (VFA) were analysed by gas chromatography (Agilent Technologies 7890A GC System, Santa Clara, CA, USA) equipped with a flame ionisation detector (GC/FID) and a polar capillary column (DB-FFAP).

Gas samples were analysed by GC (2014 Shimadzu, Kyoto, Japan) with thermal conductivity detector (TCD) (Tait et al. 2009).

NH_4-N, NO_3-N, NO_2-N and PO_4-P were determined by a Lachat QuickChem800 Flow Injection Analyser (FIA) (Lachat Instrument, Milwaukee). NH_4-N and PO_4-P were also analysed with colorimetric test kits (Merck, 114752 and 114848, Darmstadt, Germany) and measured with Spectroquant® Pharo 300 (Merck, Darmstadt, Germany).

Soluble and total Kjeldahl nitrogen (TKN) and total phosphorous (TP) were determined using sulfuric acid, potassium sulphate and copper sulphate catalyst in a block digester (Lachat BD-46, Hach Company, Loveland, CO, USA) (Patton and Truitt 1992) following by analysis with FIA (see above).

Protein analysis was done via the bicinchoninic acid (BCA) protein assay which quantifies total proteins as described elsewhere (Chang 2010).

Elemental analysis was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) analysis (Perkin Elmer with Optima 7300 DV, Waltham, MA, USA).

9.0 Solids Analysis

Total solids (TS) and volatile solids (VS) were determined by drying at 105°C for 24 hours to determine the TS. The VS was determined after 2 h in a furnace at 550°C. The TSS and VSS were determined after glass fibre filtration (Whatman, GF/C) of the sample and further processed as described for TS/VS and according to standard methods (APHA. 1998).

Temperature and pH were measured using an Oakton pH 11 Series meter (Vernon Hill, IL, USA). Illuminance (Wm^{-2}) was measured with a UV-VIS & NIR light sensor (stellarnet blue wave spectroradiometer, Warsash Scientific, Australia).

Microbial Analysis

The samples were prepared and submitted for microbial community analysis based on 16s amplicon sequencing.

10.0 DNA Extraction and Pyrosequencing

Genomic DNA was extracted from samples using FastSpin for Soil Kit (MP-Biomedicals, Santa Ana, CA, USA) according to manufacturer's protocol. 300 ng DNA of each sample was provided to Australian Centre for Ecogenomics (ACE) for 16S Amplicon sequencing by Illumina Miseq Platform using 926F (5'-AAACTYAAAKGAATTGACGG-3') and 1392wR (5'-ACGGGCGGTGWGTRC-3') primer set (Engelbrektson et al. 2010).

11.0 Data Analyis

Raw paired reads were first trimmed by Trimmomatic (Bolger et al. 2014) to remove short reads (less than 190bp) and low quality (lower than Phred-33 of 20). The trimmed paired reads were then assembled by Pandaseq (Masella et al. 2012) with default parameters. The adapter sequences were removed by FASTQ Clipper of FASTX-Toolkit (Pearson et al. 1997). The joined high quality sequences were analysed by QIIME v1.8.0 (Caporaso et al. 2010) using open-reference OTU picking strategy by uclust (Edgar 2010) at 1% phylogenetic distance and assigned taxonomy by uclust against greengenes database (McDonald et al. 2012, Werner et al. 2012). OTUs with only one read were filtered from the OTUs table by command filter_otus_from_otu_table.py in QIIME. In house script "Normaliser" (https://github.com/minillinim/Normaliser) was used to find a centroid normalized OTUs table with 40000 reads per sample.

Biological Methane Production (BMP) Tests

After finalising the wastewater treatment batch tests Section 0, the biomass grown on filtered combined wastewater was used as substrate for mesophilic anaerobic digestion to determine the biological methane potential (Jensen et al. 2011). BMP tests were done in triplicate against blanks in 160 mL serum vials, with blank methane production subtracted from replicate experiments. The mesophilic inoculum was collected from a full-scale anaerobic digester (AD) in Brisbane, Australia, operated on primary and activated sludge. An Inoculum/substrate VS ratio of 2.0 was used (Jensen et al. 2011). Bottles were flushed with N_2 for 3 min (1 L min^{-1}) and temperature controlled incubated for 40 days. Samples for ammonium and phosphate analysis were taken every at the start and the end and gas was analysed daily by GC/TCD, in the first 4 days, every 3rd day till the second week and weekly for the rest of the test. Blanks contained anaerobic inoculum and water.

12.0 Modelling and Parameter Identification

Batch Tests. A hydrolysis-limiting first order anaerobic model was implemented in Aquasim 2.1d. The initial condition used for the model estimation and simulation was TCOD of substrate. The dependent variable was methane flow on a COD basis.

The parameter estimation and analysis was done according to Ge et al. (2011). The first order hydrolysis rate coefficient (k_{hyd}) were determined following (Batstone et al. 2009).

Protein-COD was calculated according to Eding et al. (2006) (Eq.1).

$$gN\ x\ 6.25 = g\ crude\ protein;\ \ crude\ protein\ x\ 1.25 = gproteinCOD \quad (1)$$

Where;

gN = TKN (mg L^{-1}) – NH_4-N (mg L^{-1})

13.0 Parameter Estimation and Statistical Analysis.

All results reported in this work are average values from triplicate measurements and influent variability expressed as standard deviation and performance and model parameters expressed as 95% confidence interval based on a two-tailed *t*-test. Parameters regions (B_0 or f_d and k_{hyd}) were estimated by minimisation of the residual sum of squares (RSS) between the objective data and model (J=RSS). Parameter uncertainty in graphs is expressed as linear uncertainty (two-tailed *t*-test) retrieved from parameter standard error in the Fisher information matrix, and parameter confidence regions (95% uncertainty, 5% threshold) were estimated as for (Batstone et al. 2009). Appropriate F, and t-values were used for the number of parameters and degrees of freedom in all cases.

14.0 Project Outcomes

This section describes the results from the batch tests treating filtered and non-filtered red meat processing wastewater with PPB.

Red Meat Processing Wastewater

The wastewater for the batch tests was collected from a red meat processor located in NSW processing cattle only and with a capacity in the range of 1200 head per day. The wastewater was combined effluent and was collected after coarse screening and before fat recovery/primary treatment. After transport the sample was placed in a freezer (-20°C) for storage. Two batch test were carried out:

a) Using the raw combined wastewater (prior to primary treatment),
b) Using filtered wastewater (prior to primary treatment). The filtered wastewater was treated using a 0.2 mm with sieve (laboratory test sieve, Endecotts, London, England).

The characteristics of raw and pre-treated (filtered) wastewater are shown in Table *8*. The results of the elemental analysis (Inductively Couple Plasma, ICP) and the volatile fatty acids (VFA) analysis can be found in the supplementary materials (Table S1 and S2).

It is important to note that the wastewater was collected prior to secondary treatment (such as anaerobic lagoon, activated sludge lagoon). Therefore, the wastewater contained a high fraction of particulate COD and a low fraction of N present as ammonium.

The form on N is an important consideration as process performance is often assessed using uptake of ammonium N, which in this case represents only 25% of total N.

Table 8: Composition of filtered and non-filtered red meat processing wastewater (DAF influent) with standard deviation in parenthesis.

Component	Units	DAF influent (non-filtered)	DAF influent (filtered)
Total COD (TCOD)	mg/L	5906 (1700)	2660 (50)
Soluble COD (sCOD)	mg/L	1777 (37)	1900 (9.5)
VFA-COD + Ethanol-COD	mg/L	670 (60)	551 (18)
TKN	mg/L	241 (3.5)	194 (11)
NH_4-N	mg/L	62 (1.6)	50 (4.0)
NO_3-N	mg/L	n.d	1.2 (1.7)
NO_2-N	mg/L	n.d	0.7 (0.7)
TP	mg/L	27 (3.2)	25 (1.3)
PO_4-P	mg/L	19 (0.5)	26 (1.7)
COD:TN (100gCOD gTN^{-1})[a]		4.1	7.3
COD:TP (100gCOD gTP^{-1})[b]		0.5	0.9
TSS	mg/L	1.97 (0.98)	0.4 (0.3)
VSS	mg/L	1.91 (0.91)	0.2 (0.13)
pH		6.2	6.2

[a)] For every 100gTCOD the wastewater contains 4.1 and 7.3gTKN, desired is around 9,
[b)] Desired ratio is approximately 2.

Treatment of Raw Red Processing Wastewater using PPB

The treatment performance of PPB was assessed by a) SCOD, NH_4-N and PO_4-P removal efficiencies and b) the recovery potentials, assuming the separation and recovery of solid components (such as TCOD, TN and TP) in a membrane equipped photo bioreactor as described elsewhere (Hülsen et al. 2016b).

Figure 3 shows the time based TCOD, SCOD, NH_4-N and PO_4-P removal from raw red meat processing wastewater (combined effluent entering primary treatment) using PPB treatment. While 83% of VFAs were consumed during the batch tests, TCOD and SCOD were largely conserved. The conservation of COD does not mean that the tests were not successful and is not a sign for toxicity or inhibition particularly as COD is expected to be incorporated into the PPB biomass rather than removed from the batch test vial. However, a large portion of the TCOD was present as fat granules and while control tests showed a slow degradation of this material, non-dissolved fat particles (COD) may not be readily available for uptake by PPB biomass, although dissolved fat-COD is principally an excellent substrate for PPBs. Importantly, the NH_4-N and PO_4-P removal efficiencies after 100h were 92% and 25% with final effluent concentrations of 4.3 $mgNH_4\text{-}N\ L^{-1}$ and 15.8 $mgPO_4\text{-}P\ L^{-1}$. It has been established that PPB assimilate COD, N and P simultaneously. Therefore, the NH_4-N and PO_4-P removal also results in COD uptake.

The comparison of the nitrogen and phosphorous removal under different conditions shows that PPB growth is an effective mechanism for NH_4-N and PO_4-P removal. In tests with no illumination, PO_4-P was not removed and NH_4-N concentration actually increased by 50 mg L^{-1} (see Figure 3C). These results are consistent with a natural decomposition process such as anaerobic digestion (Appels et al. 2008) and confirm that 100 h is not sufficient to establish a heterotrophic anaerobic community capable of N and P removal although COD is transformed, forming VFAs. In illuminated controls with no PPB, there was some removal of NH_4-N and PO_4-P, however there was also a significant lag time (48h). This indicates 48 h may be sufficient to support growth of a phototrophic community. The wastewater with PPB inoculum removed NH_4-N and PO_4-P faster compared to the illuminated control.

Volatile suspended solids (VSS) concentration increased in the PPB tests indicating successful growth of PPB biomass. The form of COD in the vials could not be separated into PPB biomass, fat granules and other residual organics; therefore the yield of PPB biomass in terms of $gCOD_{produced}$ $gCOD_{consumed}^{-1}$ could not be measured directly. However, control tests using Ormerod medium demonstrate SCOD:NH_4-N uptake ratios of 100:10; using this ratio, the NH_4-N removal (50 mg L^{-1}) indicates at least 500 mg L^{-1} of COD was incorporated into PPB biomass (higher than the 200 mgVFA-COD removal that was measured). Furthermore, if natural decomposition of the raw material is also considered (e.g. the 50 mg L^{-1} ammonium released in the control tests), the actual ammonium conversion may have been closer to 100 mg L^{-1} and the COD uptake closer to 1000 mg L^{-1}.

The Ormerod test determined a yield of 0.94 $gCOD_{produced}$ $gSCOD^{-1}_{consumed}$ which is likely to be comparable to the yield on the wastewater.

The mass balance for nitrogen shows that NH_4-N removal was non-destructive with 98.7% of the nitrogen being accounted for through mass balancing. That is, NH_4-N removal from the wastewater during treatment was found to accumulate in the PPB biomass and crude protein (CP).

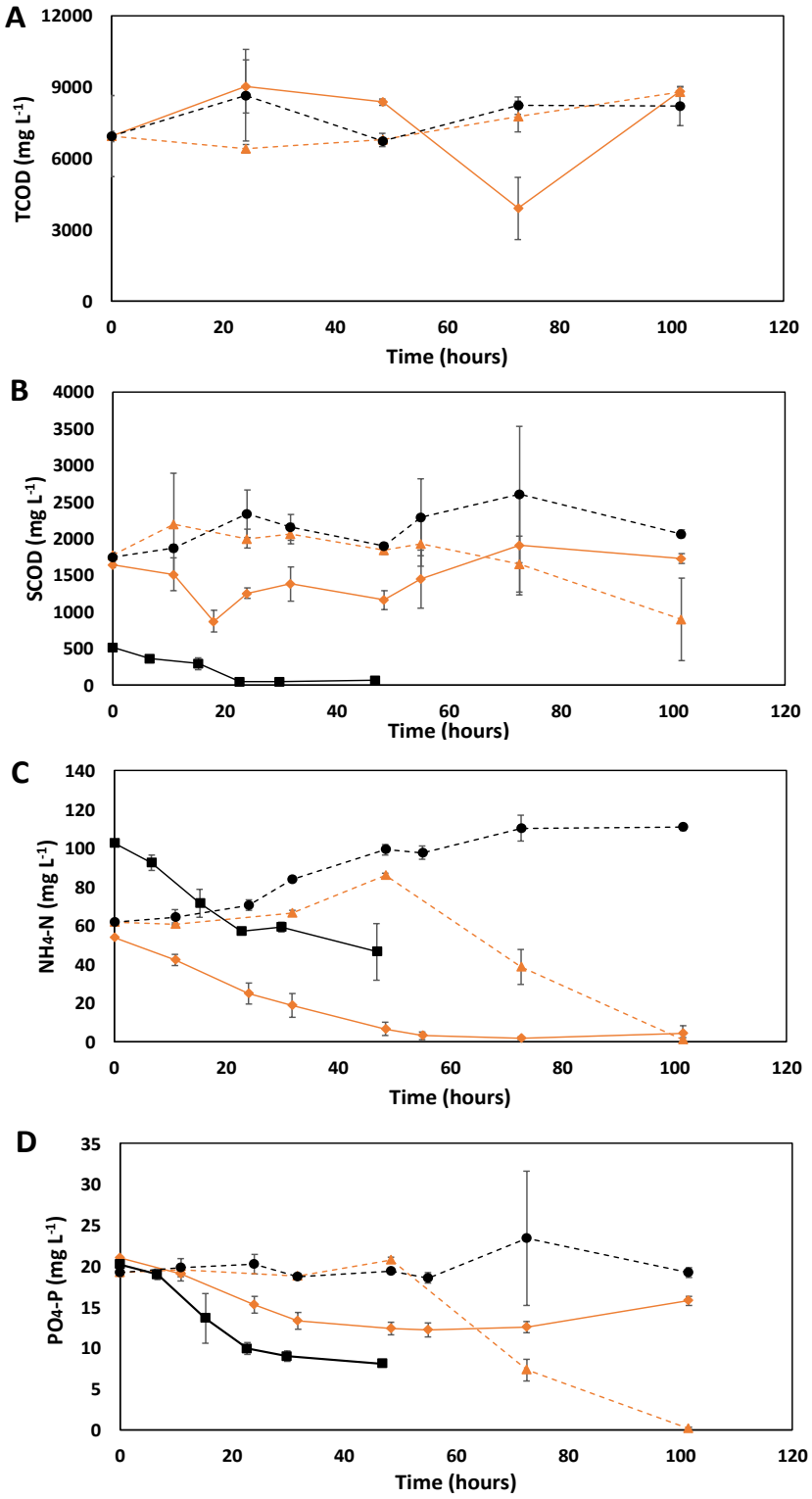

Figure 3: TCOD, SCOD, NH4-N and PO4-P concentrations over time from raw wastewater for PPB biomass + wastewater (◆), wastewater with illumination (▲, dashed line), wastewater without illumination (●, dashed line) and the PPB activity test (■).

Treatment of Pre-filtered Red Processing Wastewater using PPB

Figure 4 shows the TCOD, SCOD, NH_4-N and PO_4-P removal from pre-filtered red meat processing wastewater (filtered DAF influent) treated using PPB over time. The SCOD removal efficiency was 74%, while TCOD was again preserved – indicating that SCOD was incorporated into biomass growth.

The NH_4-N and PO_4-P removal efficiencies after 70h were 64% and 73%. Based on these tests, the effluent of a photo anaerobic membrane bioreactor would contain around 400 mg L^{-1} SCOD, 14 mg L^{-1} NH_4-N and 7.0 mg L^{-1} PO_4-P, with the remaining solid COD, TKN and TP is harvested as biomass. This removal results in 4.3g NH_4-N and 1.7g PO_4-P removed for every 100g of SCOD removed in a SCOD:N:P ratio of 100:4.3:1.7. The comparison with the dark control shows significantly lower SCOD removal (10.3%) underlining good performance of PPB treating red meat processing wastewater. Again, the dark control mobilised NH_4-N with an increase of 46% from 50 mg L^{-1} to 73 mg L^{-1} with similar background degradation behaviour expected in the PPB tests, the actual nitrogen uptake and conversion could have been closer to 80%. The illuminated control was not conducted as phototrophic community development on red meat processing wastewater was shown for the non-filtered DAF influent previously (Section 0).

The overall yield of PPB on the filtered wastewater was 0.9 ±0.1 $gCOD_{produced}$ $gCOD_{consumed}^{-1}$, indicating the potential for very high recovery. The mass balance for nitrogen shows that NH_4-N removal was non-destructive with 100.3% of the nitrogen being accounted for. As described before the NH_4-N is transformed into proteins resulting in a CP content of the harvested biomass of 0.65 ±0.02 gCP $gVSS^{-1}$. Protein content was determined independently using the BCA method as 0.57 gCP $gVSS^{-1}$ which is around 12% lower compared to the TKN method but still underlines high CP content of the biomass. These numbers are well in line with the Ormerod control indicating good performance of PPB on the tested wastewater. The results of this batch test are summarised in **Error! Reference source not found.**.

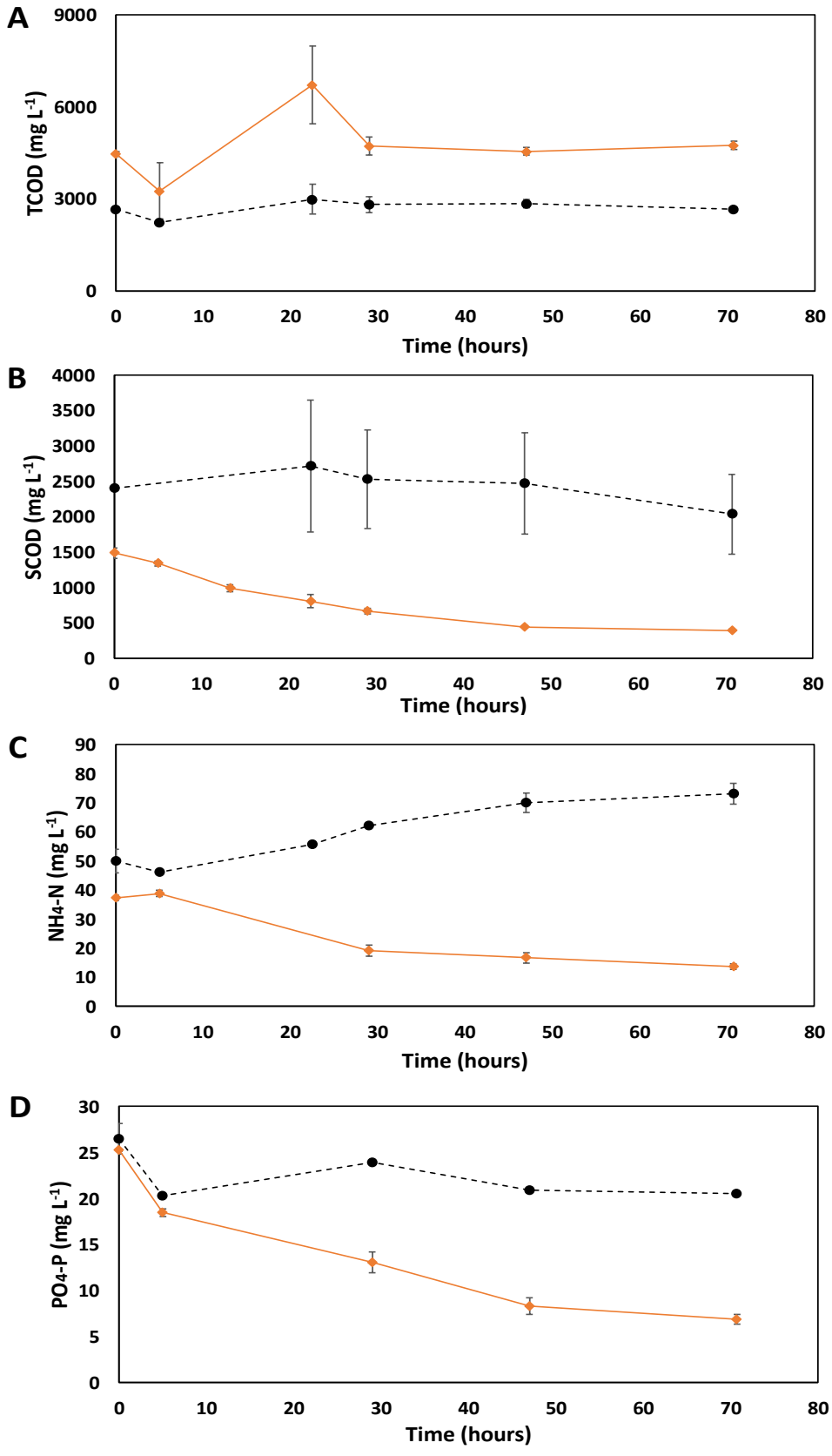

Figure 4: TCOD, SCOD, NH4-N and PO4-P removal over time from filtered DAF influent for PPB biomass + wastewater (◆) and the non-illuminated wastewater control (●, dashed line).

Microbial Analysis

The microbial community analysis shows that the filtered wastewater contains a diverse bacterial community with very low abundance of PPB, specifically 0.1% of *Rhodopseudomonas sp.* and 0.04% of *Rhodobacter sp.* which are considered as key mediator for wastewater treatment. Figure 5 shows that PPB could not develop in the dark control and this result is consistent with expectations, as light is needed as an energy source for growth.

The addition of PPB inoculum results in a dominant mixed PPB community over time with a relative abundance > 50% at the end of the test (RM_PPB_End). The presence of *Rhodopseudomonas sp.* (26%)*, Rhodobacter sp.*(20%) and *Balstochloris sp.* (7.2%) underlines the PPB importance for SCOD, NH_4-N and PO_4-P removal efficiencies. The flanking community in the PPB tests contained *Bacteriodales* and *Enterobacteriaceae,* which are commonly found in anaerobic digester communities (Wang et al. 2014) and believed to contribute to the hydrolysis of lipids, proteins and polymeric carbohydrates; and the fermentation of sugars to volatile organic acids (Rada 2015). In the PPB tests, the bacteria likely fulfil a similar role mobilising organics and nitrogen (as VFA and ammonium respectively) for subsequent uptake by the PPB.

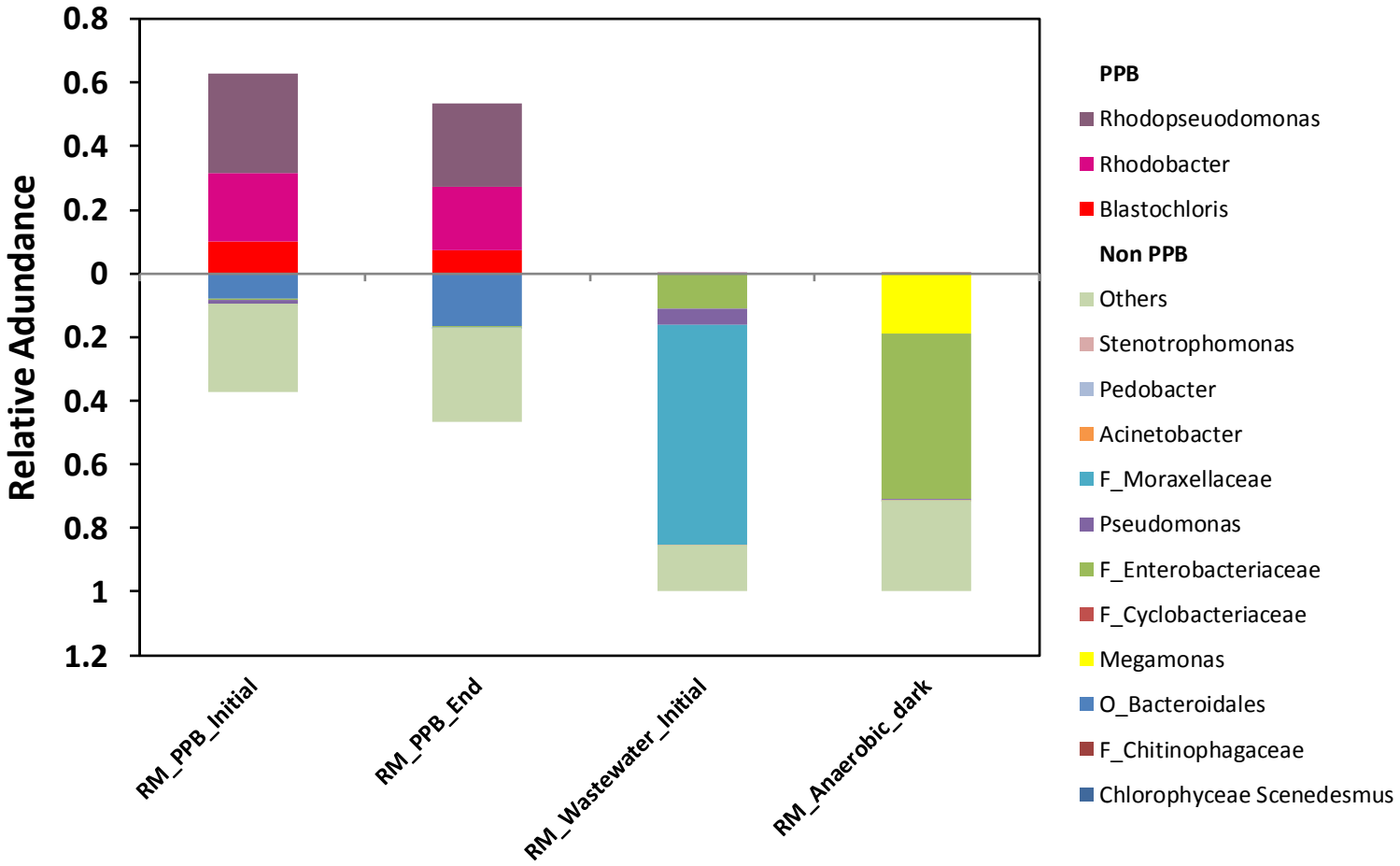

Figure 5: Relative abundance of different microbial groups at the start and the end of the batch tests, PPB are shown above the line, while other bacteria are shown below zero.

15.0 Summary of Single Cell Protein (SCP) Production

The production of high value microbial protein is a key potential benefit of the PPB process. The general composition expected for PPB biomass is presented in Table 9, showing an expected crude protein content of 65% VSS, a fat content of 33% VSS and 2% VSS as carbohydrate. The composition of PPB biomass grown during treatment of slaughterhouse wastewater is shown in Table 10 and the determined CP content is well in line with literature values (Ponsano et al. 2004, Shipman et al. 1975).

Table 9: General composition of PPB biomass and energetic value (MJ kgVSS^{-1})

Component	%VSS	MJ kgVSS^{-1}
Crude protein	64.7	10.8
Crude fat	33.1	12.5
Soluble carbohydrates	2.2	0.4
Total	100	**23.7**

Table 10: Summary of performance metrics for raw combined wastewater and filtered wastewater from red meat processing plants treatment with PPB (brackets show standard deviation).

Parameter	Unit	Filtered	Raw
TCOD removal	(%)	-4.8 (0.4)	-27.3 (5.4)
SCOD removal	(%)	73.5 (0.7)	-5.6 (5.4)
TN removal	(%)	-0.3 (3.2)	1.3 (11)
TP removal	(%)	-1.6 (7.0)	12.6 (29)
NH_4-N removal	**(%)**	**63.6 (3.1)**	**91.8 (8)**
PO_4-P removal	**(%)**	**72.7 (2.6)**	**24.8 (7)**
PPB Yield	(gCOD gSCOD^{-1})	0.9 (0.1)	-
TCOD:VSS ratio	(-)	1.5 (0.05)	2.5 (0.2)
Removal rate	(gCOD gVSS^{-1} d^{-1})	3.2 (0.5)	-
SCOD:N ratio	(-)	4.3 (0.2)	-
SCOD:P ratio	(-)	1.7 (0.03)	-
TKN lost	(%)	-0.3 (3.2)	0.5 (11)
Crude protein content	**(gCP gVSS^{-1})**	**0.65 (0.02)**	**0.61 (0.06)**

The results show that crude protein content of the PPB grown on red meat processing wastewater was 61-65% VSS and the average TCOD:VSS ratio was 1.5 with calculated yields of 0.6gVSS (PPB biomass) for every gram COD removed. This correlated to 1kg crude protein for every 2.6 kgCOD available in the wastewater. Based on the average protein, FOG and carbohydrates content an average energetic value of 23.7 MJ/kg can be determined, which is an important consideration when assessing the value as an animal feed. We note that non-hydrolysed initial protein of the wastewater may have contributed to the overall CP content, but the relative portion of slaughterhouse protein compared to PPB protein is difficult to assess. Hydrolysis products such as NH_4-N are not measured as intermediates due to direct assimilation by PPBs.

However, non-hydrolysed proteins will be harvested with the biomass or more likely will be degraded in a continuous system after the establishment of an adapted mixed community.

16.0 Heavy Metals Analysis

While the production of microbial protein is a key potential benefit of the PPB process, application as an organic fertilizer is another potential value-add end use. When considering organic fertilizer potential, the sludge classification is a critical factor impacting end use potential; and heavy metal concentrations contribute to this classification.

Allowed heavy metals concentrations for Sludge Grades A through to D are shown in Table 11. The elemental analysis of PPB inoculum and PPB samples grown slaughterhouse wastewater is shown in Table 11. PPB grown on raw combined effluent achieves the criteria for Grade A on average, however may be considered Grade B due to variability in the measured Zn. However, high Zn contamination is likely just a short-term legacy of the PPB inoculum which was originally enriched on domestic wastewater with a much higher Zn content compared to slaughterhouse wastewater. PPB biomass grown on filtered red meat processing wastewater was classified as Grade B because of elevated Copper and Zinc content. Both concentrations are high in the PPB inoculum and likely do not represent the long term concentrations of PPB grown on slaughterhouse wastewater. Continuous reactor operation is required to further investigate metals accumulation and effects on the sludge quality but the wastewater characteristics are expected to result in sludge Grade A.

Table 11: Allowed heavy metal composition of different sludge grades (A-D) and the heavy metal composition of the PPB inoculum grown on domestic wastewater as well as final composition of the solids after the red meat wastewater batch tests.

Sludge (mg kg DS^{-1})	Cd	Cr	Cu	Ni	Pb	Zn
Grade A	3	100	100	60	150	200
Grade B	5	250	375	125	150	700
Grade C	20	500	2000	270	420	2500
Grade D	32	600	2000	300	500	3500
PPB Inoculum (mg kg TSS^{-1})	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
AVG PPB Inoculum	0.00	0.00	173	n.d.	21	416
STDEV	0.00	0.00	5.0	n.d.	10	4.0
After Batch tests Raw Wastewater (mg kg TSS^{-1})	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
AVG PPB	0	0.04	58	9.3	34	184
STDEV	0	0.08	29	3.6	16	71
After Batch tests Filtered Wastewater (mg kg TSS^{-1})	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
AVG PPB	0.0	-5.0	171.0	0.0	12.2	306.6
STDEV	0.0	4.4	22.0	0.0	23.2	64.7

17.0 Biological Methane Potential (BMP) from PPB Biomass

Anaerobic digestion for methane production and subsequent energy recovery is a potential end-use option for the PPB biomass product.

The anaerobic degradability of PPB biomass grown and harvested from the filtered read meat wastewater batch test is shown in Figure 6. The cumulative methane production was measured at 381 $mLCH_4\ gVS_{added}^{-1}$ after 42 days and still increasing.

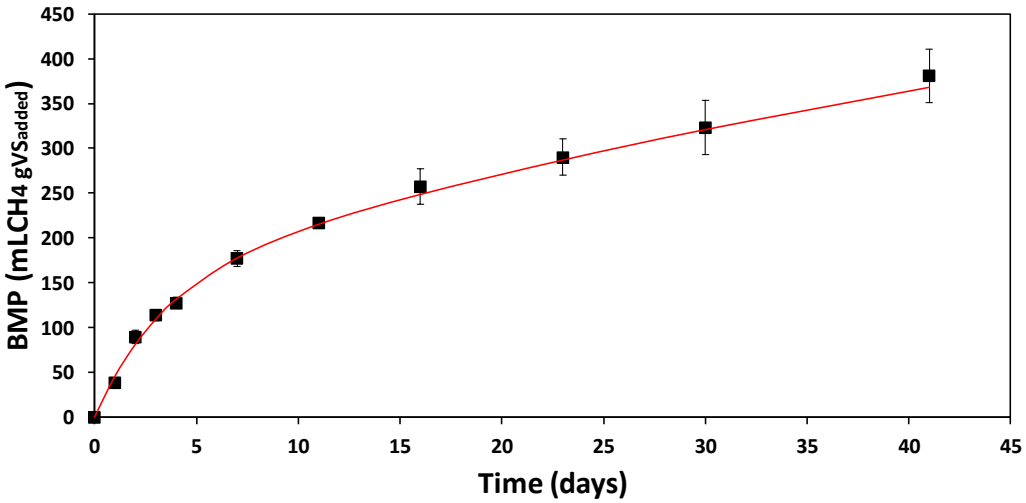

Figure 6: Cumulative methane production from the anaerobic mesophilic digestion of PPB biomass grown on filtered red meat processing wastewater.

The mesophilic curves followed characteristic first-order kinetics and were fitted with a two-substrate model. The most important outcome from this test is the total degradable fraction (fd) which was fitted at approximately 53% and indicates a significant portion of sludge waste would still be present at the end of anaerobic treatment and would require disposal – potentially at significant cost.

A second disadvantage of anaerobic digestion as a PPB end-use is the release of nutrients; 27 $mgNH_4\text{-}N\ gVS_{added}^{-1}$ and 5.5 $mgPO_4\text{-}P_{added}^{-1}$ were released during mesophilic anaerobic digestion representing 26% and 43% of N and P in the initial PPB biomass (Table 12). At this stage, it is unclear if the NH_4-N and PO_4-P could be recovered economically (e.g. as struvite, ammonium sulphate), however this option does not appear attractive due to the low recovery fractions and the low value of mineral nutrients relative to protein.

Table 12: COD, TKN and TP content of the PPB biomass and anaerobic release after 42 d with subsequent recovery potential.

PPB biomass (t=0)		
$mgTKN\ gVS^{-1}$	$mgTP\ gVS^{-1}$	$COD\ gVS^{-1}$
103.3	13.0	1.7
Released (t= 42d)		
26.5	5.5	1.0
% Released (Recovery potential)		
25.7	42.6	59.2

18.0 Economic Evaluation

Potential Value of PPB Biomass

Batch feasibility testing confirmed that protein-rich biomass can be generated while treating red meat processing wastewater using PPB. PPB performance was confirmed and all nitrogen is expected to become available for PPB growth in a continuous reactor due to i) the retention of non-soluble components with a membrane allowing time for hydrolysis and subsequent uptake by PPB, and ii) due to the establishment of an adapted microbial community.

The high protein content of PPB biomass reported in literature was also confirmed. The caloric value of the PPB biomass recovered from the feasibility testing was higher (23.7 MJ kg^{-1}) compared to values assumed in preliminary cost benefit assessment (16.8 MJ kg^{-1}) and therefore a higher product value is likely justified (i.e. a product value of \$600 $tonne^{-1}$ rather than \$400 $tonne^{-1}$). This has to be further evaluated but the composition of the biomass looks to justify a high value.

Preliminary cost benefit analysis assumed an influent COD of 10 g L^{-1} and 250 mgN L^{-1} which is higher than the meat processing wastewater used in this study (~6.0gTCOD L^{-1} with 241 mgN L^{-1} for the raw combined effluent and 2.6 gTCOD L^{-1} with 194 mgN L^{-1} for pre-filtered wastewater). Therefore, the potential yields and value recovery per ML of wastewater has been revised accordingly.

Figure 7 shows the potential value recovery from 1 kL of wastewater, including comparisons for average influent concentrations of 10, 6 and 2.6 gTCOD L^{-1} with corresponding N concentrations of 250, 241 and 194 mgN L^{-1}. This comparison still demonstrates that the potential value of PPB generated from meat processing wastewater is 3-5 times greater than the value of methane energy or mineral nutrients that could be recovered.

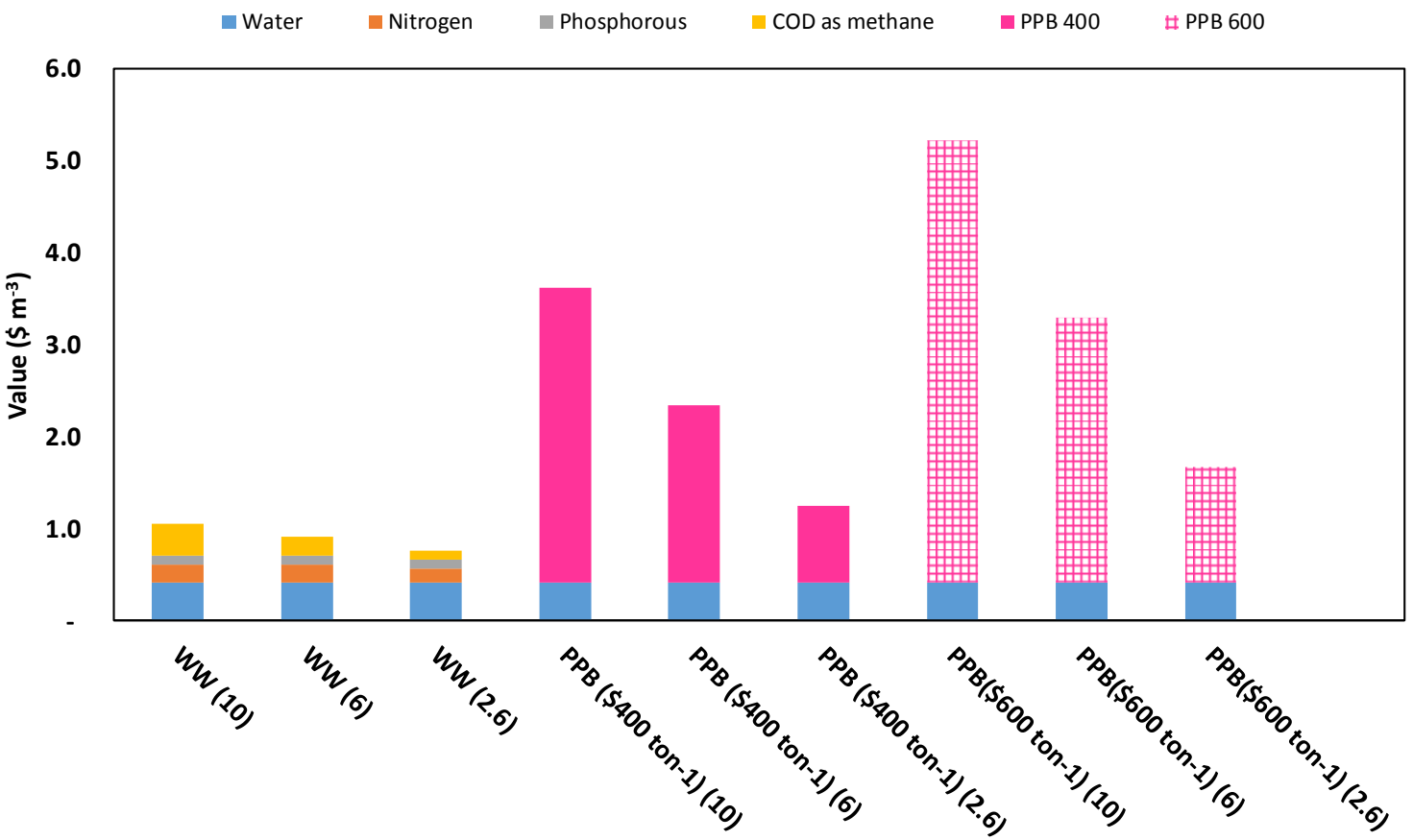

Figure 7: Potential value of 1.0 m^3 of red meat processing wastewater at different COD influent concentration (10, 6 and 2.6 gTCOD L^{-1}) when recovering all resources (WW), selling the biomass at \$400 t^{-1} and \$600 t^{-1}.

Importantly, the market value of PPB is still unclear, \$400-600 tonne^{-1} is been used as a baseline value (due to crude protein content), however if the protein and amino acid profiles are more favourable, the value opportunity may be higher. **Error! Reference source not found.** shows the protein and amino acid content of PPBs compared with competing products. This comparison demonstrates some potential to use PPB as an alternative protein source. However, this is based on the assumption that this value can be realised, and requires further investigation.

Table 13: Protein and amino acid content and price of commercial feed protein sources in comparison with PPB and algae grown in this study and estimated value.

Component	Fishmeal	Rendered Meat Meal	Poultry by-product meal	Blood meal	soybean meal	PPB
Total Protein	**64.5**	**55.6**	**59.7**	**89.2**	**50**	**63.7**
Leucine	4.48	2.85	4.11	10.82	3.63	3.4
Valine	2.77	2.52	2.86	7.48	2.55	2.5
Arginine	3.82	3.6	4.06	3.75	3.67	2.3
Phenylalanine	4.35	4.35	2.99	3.97	4.2	2.2
Threonine	2.31	1.64	0.94	3.76	1.89	2.1
Lysine	4.72	2.93	3.06	7.45	3.08	2.0
Isoleucine	2.66	1.64	2.3	0.97	2.14	1.9
Methionine	2.31	1.25	1.94	2.32	1.43	1.0
Histidine	1.45	0.89	1.09	5.14	1.22	1.0
Tryptophan	0.57	0.34	0.46	1.04	0.69	-
Price AUD ton^{-1} (dry)	1860- 2280	400-600	400-600	870-1160	390-440	**350**
Price in CP kg^{-1}	1.19 - 1.47	0.22-0.33	0.24-0.36	0.78-1.04	0.20-0.22	**0.22**

Prices from:
https://www.google.com.au/search?q=price+fishmeal&ie=utf-8&oe=utf-8&client=firefox-b-ab&gfe_rd=cr&ei=jdZYV5KVH6fM8ge0wL3ABQ
http://future.aae.wisc.edu/data/weekly_values/by_area/3191?tab=feed
http://future.aae.wisc.edu/data/weekly_values/by_area/3188?tab=feed
https://www.google.com.au/search?q=price+fishmeal&ie=utf-8&oe=utf-8&client=firefox-b-ab

Comparison of Red Meat Wastewater Treatment Options

Appendix C. contains a series of case studies designed to compare the economic potential of PPB to existing and developing options for treating red meat processing wastewater. Outcomes from the case studies are summarised in this section.

A comparison of the capital and operating costs of the different treatment options evaluated in the case study report and an update including the newly gained insights is shown in Table 14. The costing information is not intended as a detailed feasibility analysis; it is intended as a preliminary comparison of the novel PPB in a PAnMBR with current and emerging technologies for red meat processing wastewater. Furthermore, it gives an indication of the relative contributions of the organic removal and nitrogen removal steps to capital and operating costs of the different technologies. Due to the novelty of the PPB process the capital costs of the reactor can only be estimated. At this point we consider the selected values as representative but future developments might change the capital investment.

The feasibility analysis showed, that based on the assumptions, capital costs of a PPB process are not excessive and are comparable to existing technologies and potentially less expensive than conventional biological nutrient removal technologies.

Operational costs for a PPB process include illumination intensity at 1 kWh m^{-1}, however lower intensity of 0.1 kWh m^{-3} (assuming 20 $m^{-2}m^{-3}$ surface area) are more realistic for full-scale and this would improve the comparison, however this is still to be confirmed. For PPB to be economically feasible the biomass has to be marketed as a high value organic fertiliser and/or as protein-rich feed additive. Based on this report, PPB would have a similar or higher value and similar applications to existing rendering products such as meat and bone meal, therefore PPB could potentially be marketed through the same supply chains; decreasing the risks associated with developing a new market for the product. The production of organic fertiliser as well as feed additive was based on wastewater containing 6gTCOD available with 250mgN L^{-1} which would generate a value of $1.92 m^{-3} treated (Figure 7:) at $400 $tonne^{-1}$, resulting in an annual revenue of $830,400. A value of $600 $tonne^{-1}$ would increase the revenue to $1,245,600 per year. Based on an estimated capital investment of $4,109,000 the payback times are 3-5 years. This is based on a reactor volume of 1730 m^3 which would require an HRT of 1 day. This has to be confirmed in continuous reactor operation.

PPB technology is also able to integrate with some existing treatment processes, such as covered anaerobic lagoons. The PAnMBR reactor could be placed in the main line prior to the CAL – with the excess COD then sent for polishing in the CAL or the PAnMBR could be located after the CAL to treat the CAL effluent and a portion of the raw wastewater (~25-30%). In both cases sufficient COD can be supplied to remove N and P in the PAnMBR. This option can utilise present infrastructure and therefore reduce the capital costs. This configuration includes the production of biogas in the CAL and the production of PPB product in the PAnMBR, resulting in a potential payback time of around 4-5 years.

If PPB is integrated with a side-stream AD process and secondary nutrient removal, the capital costs would increase substantially. At the same time, anaerobic digestion destroys the PPB biomass and reduces the value. This option does not appear attractive. However, energy recovery through AD is not the target application of the PPB biomass product and therefore the poor economics of this option do not impact project viability.

Table 14: Comparison of Nitrogen Removal Case Studies

Parameter	CAL + BNR	CAL + BNR (WAS recycle to CAL)	CAL + anammox	AnMBR + anammox	High Rate SBR +AD + anammox	PPB ($400 tonne^{-1})	PPB ($600 tonne^{-1})	PPB + AD +nutrient removal	CAL +PPB (with bypass)	CAL +Raceway Pond***
Capital Costs										
Organic Removal	$1,858,000	$2,026,000	$2,303,000	$5,816,441	$3,598,000	$4,109,307	$4,109,307	$11,049,947	$1,658,000	$2,303,000
Nitrogen Removal	$3,289,000	$3,418,000	$549,000	$607,000	$1,178,000			$642,00	$4,082,000	$398,000
Total Capital	**$5,147,000**	**$5,444,000**	**$2,852,000**	**$6,423,00**	**$4,776,000**	**$4,109,000**	**$4,109,000**	**$11,692,00**	**$5,741,000/$ 4,109,000***	**$2,701,000**
Operating Costs										
Organic Operating	-$577,000	-$559,000	-$725,000	-$805,000	-$500,000	-$830,400	-$1,245,600	-$542,000	-$522,000	-$725,000
Nitrogen Removal Operating	$132,000	$204,000	$51,000	$48,000	$103,000			$107,000	-$830,400	$146,000
Total Operating	**-$445,000**	**-$355,000**	**-$674,000**	**-$757,000**	**-$397,000**	**-$830,400**	**-$1,245,600**	**-$435,000**	**-$1,352,400**	**-$579,000**
Payback (years)	**11.6**	**15.3**	**4.2**	**8.5**	**12.0**	**5.3**	**3.5**	**26.9**	**4.2/3.0***	**3.8**

* no infrastructure present/ presence of CAL and Cogeneration. And ($400 tonne^{-1}).

19.0 Conclusions/ Recommendations

Key Challenges and Knowledge Gaps for Application of PPB to Red Meat Wastewater

The key challenges and knowledge gaps as identified in the feasibility study are listed below whereby new insights gained during the project are included and discussed. Specifically;

External COD supply to remove TN and TP to below discharge limits

Our experience with PPB treating diluted domestic wastewater can be translated to the red meat wastewater. A major challenge for domestic wastewater treatment is the unsuitable SCOD:N:P ratio. Additional COD e.g. in the form of methanol has to be added to achieve low TN and TP effluent concentrations. The COD:N:P ratio of the wastewater is crucial. Adding external COD is expensive and challenges the economic feasibility of the PPB treatment process. However, the COD of red meat wastewater is high and external COD supply is not needed. In fact, the opposite is true. COD may be present in excess and research has to determine the COD:N:P ratios of PPB treating red meat processing wastewater.

This study: External COD is not required. Treating full strength red meat processing wastewater with 10 gTCOD L^{-1} and 250 mgN L^{-1} would lead to excess COD. However, the pre-treatment in a DAF unit could reduce COD significantly with limited impact on nitrogen resulting in an improved COD:N ratio. The results indicate that a PPB process can simultaneously reduce SCOD, N and P in treated wastewater >70%..

COD:N:P ratios of the red meat wastewater

Although the COD:N:P ratios in red meat wastewater are favourable for complete N and P removal, there may be excess COD and N and/or P can become limiting. Bacteria need macronutrients to grow. If all N and P is consumed residual COD might be present in the effluent. This depends on the daily wastewater composition. However, over time the development of a synergistic community is expected that balances the COD, N and P uptake. Alternatively, PPB could be applied to CAL effluent. This would allow excess COD in the wastewater to be recovered from the CAL as methane, offsetting energy consumption at the slaughterhouse. If needed a fraction of the raw wastewater could bypass the CAL to balance the COD:N:P ratio. Therefore, excessive COD is not likely to impact viability of the technology and may actually facilitate energy recovery.

This study: As discussed above nitrogen can become limiting. Continuous reactor operation of pre-treated (DAF) wastewater is required to determine the extent. In the case of nitrogen limitation and access COD several combinations can be tested. As mentioned above a) CAL effluent and raw influent can be combined to supply NH_4-N from the CAL and COD with N from the influent, b) primary influent to grow as much valuable PPB as possible, removing the nitrogen and P leaving low amounts for post anaerobic treatment e.g. in a CAL.

Illumination intensity has to be reduced to a minimum to save energy

Long term (>2years) lab-scale reactor operation was done with IR light at illumination intensities of 50W m^{-2} to prove the concept. Our experience clearly showed that 20W m^{-2} are possible and literature values are as low as 7.3 W m^{-2} (Basak and Das 2009), this would decrease the operational cost considerably and will be part of this study.

This study: The batch tests in this study where illuminated with 10 Wm^{-2} which seems possible and will reduce the overall operational costs.

Thickening of biomass and harvesting

Long term lab-scale reactor operation was conducted using suspended biomass and required a membrane for biomass retention. Harvesting of PPB and thickening from suspended biomass to practical concentrations (>1%) is not economically feasible (also a major challenge for algae and cyanobacteria). Therefore, the second generation PPB reactor will target attached growth on illuminated surfaces. We have previously measured solid concentrations on indirect illuminated surfaces of up to 11% as VS (or 110g kg^{-1} wet). Similar or better results are expected on directly illuminated surfaces which will reduce the thickening costs and make further treatment feasible. The concentration of biomass after collection from the PAnMBR with attached growth on illuminated surfaces has to be tested.

This study: Biomass harvesting is beyond the scope of the current project, but should be a focus area in follow up research. An early prototype utilising attached growth will be started in August 2016 as part of another jointly funded project (CRC for water Sensitive Cities (project 2.1) and the Smart Water Fund (project 10OS-023). The outcomes can be directly translated to red meat processing wastewater.

Nutrients release during anaerobic digestion of PPB biomass

Anaerobic digestion can be applied as a strategy for energy recovery from PPB biomass, but will also mobilise nutrients. Previous results show a high release and recovery potentials and this would suggest high secondary treatment costs. However, anaerobic digestion of PPB biomass is not the desired application. Harvesting the biomass from the illuminated surface and optional additional thickening is expected to produce high quality pellets with balanced elemental composition including the majority of the COD, N and P removed from the wastewater.

This study: The economic evaluation demonstrates that anaerobic digestion of PPB biomass would result in reduced value recovery and would require secondary treatment of the digester effluent, which further reduces the value proposition. This process configuration is not recommended for further development.

The value of PPB as organic fertiliser and/or animal feed additive

It will be crucial to determine the characteristics of the PPB biomass to determine application potential and value. The biomass needs to be graded based on pathogens and heavy metal content. The energetic value has to be determined and steps to utilise the biomass as feed additive have to be determined. The value of the product is critical to the payback time and overall feasibility.

*This study:: This project has confirmed high protein (>60%) and energy content of the PPB biomass (>20MJ $kgVSS^{-1}$) which increases the potential value and makes application as feed or feed additive appear attractive. The pathogens have to be determined as part of a product characterisation prior of feed trials. Suitable pre-treatment and pathogen destruction such as heat sterilisation will be tested as part of another project (Australian Pork Limited (*Project No: 2014/534.05*) and can be directly applied for red meat waste streams.*

PPB biomass characteristics compare well with conventional feed/feed additives, however market value still requires validation. Livestock feed trials will be important to establish and confirm market value, with trials on pigs and fish being planned through broader collaborative projects. This will determine possible substitution of current feed and determine the value of PPB biomass. The heavy metal content of PPB biomass grown on the wastewater (with PPB enriched on domestic wastewater) is likely to achieve a Grade A classification in terms of contaminants, enabling a broader range of end-use applications.

Additional challenges identified for diluted wastewater treatment are not relevant for red meat wastewater. However, the overall treatability/degradability of the wastewater to PPB has to be studied. Problems might arise from the FOG content in the wastewater. Potential inhibition and fat accumulation have to be determined. PPB are expected to be able to utilise the FOG, once broken down to smaller units (LCFA→VFA). The FOG content will depend on the primary treatment performance. The degradation and utilisation of solid COD has to be determined.

Conclusions

The outcomes of this project demonstrate PPB technology for red meat processing wastewater treatment is a new and potentially disruptive technology for generation of value-add products that could substantially change the economics of wastewater treatment. The major findings of the project are:

- Combined wastewater entering primary treatment (such as dissolved air floatation) can be treated using PPB achieving 92% NH_4-N removal and 25% PO_4-P removal whereby TN and TP are persevered in the biomass. The portion of COD converted to protein biomass could not be determined due to the particulate nature of COD in the feed.
- When combined wastewater was pre-filtered to remove particulates, PPB treatment resulted in 74% SCOD, 64% NH_4-N and 73% PO_4-P being removed from the wastewater and converted to microbial protein.
- Time series data showing nutrient removal during treatment indicates process retention time of 2 days or less can be achieved through optimisation.
- The PPB biomass product had a crude protein content above 60%, confirming potential to generate a value-add product.
- Using a photo anaerobic membrane bioreactor (PAnMBR), a PPB process would generate microbial protein product with higher crude protein and energy content compared to existing byproducts (Meat and Bone Meal) or competitor products (Cotton Seed Meal). This could generate a potential product stream with high value ($400-600 t^{-1}). This strategy is strongly recommended for further development.
- Anaerobic digestion of PPB biomass for energy recovery will results in lower value recovery and will require secondary treatment of the digestate, further reducing the value proposition. This strategy is not recommended for further research.
- PPB biomass can be anaerobically digested to generate renewable biogas energy. Methane yields are higher than existing sludge treatment processes, however revenues are lower and this option creates a requirement for secondary effluent treatment.

- The capital costs of the PAnMBR process without dedicated solids treatment is approximately 40% more expensive compared to developing configurations such as CAL+ anammox and CAL + Algae Pond. However, capital costs are lower compared to AnMBR + anammox, High rate SBR + AD + anammox, CAL+BNR or CAL+BNR (WAS recycle).

Future research will focus on the continuous lab-scale reactor operation with focus on COD:N:P ratios but also on the process parameters such as volumetric loading rates, sludge retention times and hydraulic retention times. These parameters will determine the size of the PAnMBR and are crucial for capex confirmation.

At the same time, an interdisciplinary team including, feed producers (extrusion, drying and sterilisation), nutritionists and farmers (for now aquaculture and pigs) is actively working on the product development.

Based on the economics it can be concluded that PPB technology offers high potential. However, it also includes some risks. The outcomes of this feasibility report are summarised in a SWOT analysis:

SWOT Analysis
PPB for red meat wastewater treatment

Strengths	Weaknesses
• Non-destructive treatment. • Organics, Nitrogen and Phosphorous recovered. • Potential for new revenue through organic fertiliser or protein rich feed product. • Single step treatment that produces effluent suitable for discharge. • Larger programme enables synergy, strong background knowledge. • Pilot work progressing in larger programme.	• Only batch and pilot scale at this point. • Energy consumption. • Response to solid COD is unknown. • Response to FOG is unknown. • Optimal COD:N:P ratio unknown. • Application and value of PPB biomass is uncertain. • New market needed. • Legislation around PPB biomass use.
Opportunities	**Threats**
• Efficient wastewater treatment with strong value-add potential. • Increase wastewater value 3-4x. • Huge image aspect. • Team is world leader in the area. • Unique IP able to be utilised. • Reduced wastewater discharge costs due to high removal efficiencies. • Development of cross industry linkages to improve sustainability.	• Loading rates cannot be achieved – blowing out capital costs. • Wastewater treatment not efficient, no use of solid COD. • Strong international research competition emerging. • Energy consumption too high. • PPB biomass potentially not suitable as organic fertiliser.

• Costs savings for protein source. • Payback times ~4 years. • Fits with established marketing channels e.g. MBM.	• PPB biomass potentially not suitable as feed additive.

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21.0 Appendix A: Supplementary Data

Table S1: Total, dissolved and solid elemental composition of raw and filtered combined wastewater

Raw Waste Water		Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn
Total	**AVG**	0.0	0.0	0.0	0.1	81.4	0.0	0.2	0.0	0.2	8.7	88.1	29.0	0.5	0.0	247	0.1	54.5	0.5	63.7	0.0	0.0
	STDEV	0.0	0.0	0.0	0.0	5.8	0.0	0.0	0.0	0.0	0.5	0.7	0.4	0.0	0.0	0.9	0.0	0.7	0.2	2.6	0.2	0.0
Dissolved	**AVG**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	44.9	13.4	0.0	0.0	130	0.0	22.4	0.1	19.9	0.0	0.0
	STDEV	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	1.4	0.0	0.3	0.0	0.5	0.0	0.0
Solid	**AVG**	0.0	0.0	0.0	0.1	81.4	0.0	0.2	0.0	0.2	8.7	43.2	15.6	0.4	0.0	117	0.1	32.1	0.4	43.8	0.0	0.0
	STDEV	0.0	0.0	0.0	0.0	5.8	0.0	0.0	0.0	0.0	0.5	0.4	0.6	0.0	0.0	2.1	0.0	1.0	0.2	2.6	0.1	0.0
Filtered Waste Water																						
Total	**AVG**	0.0	0.0	0.0	0.0	11.6	0.0	0.0	0.0	0.1	27.4	107.4	27.9	0.2	0.0	231	0.0	52.1	0.2	50.0	0.0	0.0
	STDEV	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.2	4.5	1.4	0.0	0.0	12.3	0.0	2.7	0.0	2.8	0.1	0.0
Dissolved	**AVG**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	44.9	13.4	0.0	0.0	130	0.0	22.4	0.1	19.9	0.0	0.0
	STDEV	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	1.4	0.0	0.3	0.0	0.5	0.0	0.0
Solid	**AVG**	0.0	0.0	0.0	0.0	9.0	0.0	0.0	0.0	0.1	15.7	58.6	13.4	0.1	0.0	107	0.0	25.9	0.0	26.7	0.0	0.0
	STDEV	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.2	5.6	1.9	0.0	0.0	16.6	0.0	3.4	0.0	3.6	0.1	0.0

Table S2: VFA-COD profile of raw and filtered combined wastewater

Raw Waste Water	Ethanol	Propanol	Butanol	Acetic acid	Propionic acid	iso-Butyric acid	Butyric acid	iso-Valeric acid	Valeric acid	Hexanoic acid	Total VFA-COD
AVG	3.3	0.0	0.0	201.5	315.1	26.1	64.7	48.4	11.2	3.2	673.6
STDEV	5.7	0.0	0.0	16.5	26.3	2.1	4.9	3.9	0.6	0.3	60.1
Filtered Waste Water											
AVG	2.9	0.0	0.0	185.3	252.8	15.5	37.7	34.2	13.8	8.6	550.7
STDEV	5.1	0.0	0.0	6.7	8.9	1.4	1.9	1.0	1.6	2.3	18.2

22.0 Appendix B: Literature Review Purple Phototrophic Bacteria

Introduction to Purple Phototrophic Bacteria

Purple phototrophic bacteria (PPB) are commonly distributed in the natural environment in soil, fresh water, marine environments and wastewater, and can be readily isolated from these sources [22].

PPB generate chemical energy from light rather than from other chemicals [23]. This is a prerequisite for high biomass yields and avoids gaseous emissions due to product formation. The capability to generate energy from light is related to the presence of either chlorins (Chl) or bacteriochlorins (BChl) which are photosynthetic pigments that occur in various phototrophic organisms. Different pigments allow the organism to utilise different light spectrums. Table 15 gives an overview of BChls and Chl and the absorption maxima. Furthermore, the carotenoids give the PBB culture specific colour, ranging from yellow, orange to red [24]. Anoxygenic photosynthesis, where light energy is captured and converted to ATP without the production of oxygen, is a key mechanism in the proposed PPB process and has been extensively studied and reviewed [24]. Further readings about the biochemistry and molecular structures of the light harvesting complexes (LHC) can be found elsewhere [25, 26].

Table 15: Absorption maxima of different chlorins.

*BChl and Chl	Wavelength or wavelength range of absorption maxima (nm)
BChl *a*	375, 590, 805, 830-911
BChl *b*	400, 605, 835-850, 986-1035
Chl *c*	457-460, 745-755
Chl *d*	450, 715-745
Chl *e*	460-462, 710-725
BChl *g*	375, 419, 575, 788

Adapted from [27].
*BChl *a*, *b*, and *g* are bacteriochlorins, Chl *c*, *d* and *e* are chlorins.

Importantly, PPB contain BChl a and/or BChl b, these pigments enable them to absorb light in the near infra-red (NIR) and this capability is not shared by other phototrophs such as algae or cyanobacteria [28]. Therefore, IR light provides PPB with a distinct competitive advantage and can be used to select for phototroph communities of PPB. The capability of PPBs to utilise IR is also a distinct operational advantage as IR light from light emitting diodes (LEDs) can save up to 70% of the power requirements compared to white light [28], needed for algae growth. Finally, since phototrophs utilise organics for growth rather than CO_2 (photosynthetic), the light input per gram biomass is far less.

Application of PPB for Wastewater Treatment

PPB have high potential in the treatment of wastewater due to removal of COD [29] but also removal of phosphorous through polyphosphate (polyP) formation [30], removal of NO_3-N by denitrification [31, 32], removal of NH_4-N by assimilation [33] and odour reduction due to H_2S assimilation and oxidation [34].

At the same time, valuable products can be produced such as; polyhydroxybutyrate (PHB) [35] bio-hydrogen [36], oxycarotenoids [37] and the PPB biomass itself with potential applications as organic fertiliser [38] or animal feed [39].
Several PPB strains have been isolated from various sources for wastewater treatment. Table 16 shows a variety of studies treating wastewaters with different species of PPB. These studies show the potential for PPB in wastewater treatment. However, many of these studies were not based on realistic real word conditions and utilised axenic cultures grown in predefined media as inoculum (i.e. cultures with only the desired organisms). Most of the experiments were conducted with synthetic wastewater or sterilized influent which avoids competition with other organisms and does not represent a true industry application.

However, PPB have been applied to non-sterile wastewater, achieving satisfying COD removal from sardine processing wastewater by *Rhodovulum sulfidophilum* [29, 40]. Kantachote, Kornochalert [41] used *Rhodopseudomonas palustris* to remove COD and H_2S from a mix of raw rubber sheet wastewater and fermented plant extracts. COD removal from tuna condensate and a mix of tuna condensate and shrimp-blanching water by *Rhodocyclus gelatinosus* grown in G5 medium was reported by Prasertsan, Choorit [42]. These wastewaters contain high concentrations of COD (7.0 up to 60 g L^{-1}) but low N and P.

PPB have been applied successfully for domestic wastewater in batch tests [43] as well as in continuous lab-scale photo anaerobic membrane bioreactors at ambient [44] and cold temperatures [45]. PPB were able to remove organics, nitrogen and phosphorous simultaneously, in one step to below discharge limits (COD <100 mg L^{-1}, TN <10mg L^{-1} and TP <1mg L^{-1}). For every 100 g of SCOD, 8 g of NH_4-N and 1.3 g of PO_4-P are assimilated, resulting in a SCOD:NH_4-N:PO_4-P substrate ratio of 100:8:1.3. This concept was proposed as a new platform for wastewater treatment of the future, including the recovery of heat energy and fertilisers due to non-destructive assimilative treatment [46]. However, publications describing the full-scale application of PBB to treat wastewater are currently limited and this creates some uncertainty.

Table 16: Summary of wastewater treated with PPB.

Wastewater	Pre-treatment	$COD_{removed}$	PO_4-P $_{removed}$	NH_4-$N_{removed}$	HRT	Light	PPB	Ref
-	-	%	%	%	d	Lux	-	-
Noodle Processing WW	-	90			6-10		Rps. Palustis and Rba. Blasticus	[47]
Tilapia fish processing WW	filtered, pasteurized	43		22.5 (TN)	3-7	1,400 ± 200	R. gelatinosus	[48]
Sardine processing WW	-	71			5	2500	R. sulfidophilum	[29]
Sardine processing WW	settling	77			5	2500	R. sulfidophilum	[29]
Latex processing WW	autoclaving	57			1.7	3000	R. gelatinosus	[49]
Swine WW	autoclaving	90 (diluted), 50 (undiluted)	58		6	4000	Rps. palustris	[50]
Tuna condensate	1:10 dilution	78			5	3000	R. gelatinosus	[42]
Tuna condensate and shrimp blanching water	-	86			5	3000	R. gelatinosus	[42]
Food processing WW	-	518(MBR), 48 (SBR)			10	IR	mix	[51]
Olive mill WW	dilution	33 CL, 31 (dark /light)				200 W m^{-2}	R. spaeroides	[52]
Poultry slaughterhouse WW	filtered, pasteurized	91			10	4000 ± 500	R. gelatinosus	[53]
Pharmaceutical WW	add $(NH_4)_2SO_4$ and yeast extract	80			5	6000	R. sphaeroides	[54]
Sardine processing WW	-	85			5	2500	R. sulfidophilum	[40]
Synthetic sewage WW	-	89	77	99 (NO3-N)	2	-	R. sphaeroides Rps. palustris	[34]
Latex rubber sheet WW	add $(NH_4)_2SO_4$ and nicotinic acid, centrifuged	90			4	3000	Rps. blastica	[55]
Latex rubber sheet WW	filtering	80			3	-	R. palustris	[41]
Sulfate containing food industry WW	-	90			3-10	45 W m^{-2}	Mix	[56]

End Use and Value of PPB

PPB contain a variety of useful products such as, vitamins, carotenoids, ubiquinone [57] and proteins [56]. Carotenoid pigments are another potential valuable bio-product. Carotenoids can be used commercially as vitamins, antioxidants and for cancer chemoprevention [58]. While these compounds are potentially very high value, additional extraction and purification steps are required.

In addition, the PPB biomass itself can be used, without extraction of specific components. The use of PPB as organic fertiliser was supported by Xu [38] who reported improved soil quality, growth and yield of crops. Kobayashi and Tchan reported increased production of citrus fruits when PPB were applied as organic fertiliser [39].

Another potentially important application is PPB as protein rich feed additive. The PPB biomass was reported to be an excellent food additive for fish farming, also increasing the survival of fish [39]. The same study reported increased egg production in hens with PPB biomass as feed additive. Ponsano, Pinto [37] reported the use of PPB as poultry feed.

The composition of PPB is shown in Table 17 and is comparable with meat and bone meal (MBM) produced in many slaughterhouses. MBM is an established product of the rendering industry and primarily marketed as a feed additive. PPB biomass has similar potential as a single cell protein and feed additive [59]. Single cell protein is an emerging category of waste derived products gaining substantial traction internationally. The production of single cell protein from cultivated microbial biomass is considered as an alternative proteaceous food source for the future [59]. If PPB biomass can be utilized effectively, this could substantially shift the economics of wastewater treatment. The average composition of PPB and MBM is shown Table 17. The value of MBM reported in the MLA co-product market report for September 2015 is approximately \$670 t^{-1} and has been relatively stable for the past 2 years, however a more conservative value of \$400 t^{-1} will be used this report.

Based on Table 7, PPB has an average protein content of 62.9% and an average energetic value of 16.8 MJ kg^{-1}. These values were used to compare prices for feed additives based on the dry matter (\$ $kgDM^{-1}$), energetic value (\$ MJ^{-1}) and crude protein (CP) costs (\$ kg CP^{-1}).

Table 17: General composition of PPB and MBM and energetic values.

	R. capsulatus[1]		Rps. Gelatinosa[2]		R. gelatinosus[3]		MBM[4]	
	% DM	MJ kg^{-1}	% DM	MJ kg^{-1}	% DM	MJ kg^{-1}	% DM	MJ kg^{-1}
Crude protein	60.9	10.2	65	10.9	62.8	10.5	50	8.4
Crude fat	9.9	3.7	n.d	3.7	0.5	0.2	10	3.8
Soluble carbohydrates	20.8	3.5	n.d	3.5	25.6	4.3	n.d	-
Crude fiber	2.9	-	n.d	-	n.d	-	n.d	-
Ash	5.3	-	n.d	-	4	-	34	-
Total	-	17.4	-	18.1	-	15	-	12.1

adapted from [1] [60], [2] [61], [3] [37], [4] [62], n.d = not determined.

Table 8 shows prices for common feed additives or standalone fodder. Based on this table, average costs were calculated, resulting in; 32.8 $cent kg DM^{-1}, 2.2$cent MJ^{-1} and 1.7 $ kg CP^{-1}. MBM is included in the table for comparison, but is not included in the calculation. Table 8 also shows a comparison of PPB and the corresponding energy/protein costs based on different values of PPB biomass. Based on common fodder prices, the values of PPB biomass should be around $400 t DM^{-1} ($0.40 kg DM^{-1} and $0.024 MJ^{-1}). However at $400 t^{-1} the CP price is $0.6 kg CP^{-1} which is 65% lower compared to the average CP price and therefore appears conservative.

Considering a variety of other protein meals such as; soybean meal; $350 – 480 m^{-3}, feather meal; $400 m^{-3}, and poultry meal $650-775 m^{-3} a price of $400-600 t^{-1} for PPB seems possible (Source: [63]).

Table 18: Overview of different feed sources with metabolisable energy and crude protein (CP) content and allocated costs.

Source	DM (%)	Metabolisable energy (MJ kg DM^{-1})	CP (% DM)	$ t^{-1}	$cent kg DM^{-1}	$cent MJ^{-1}	$ kg CP^{-1}
Barley*	90	12	12	230	25.6	2.1	2.1
Pasture hay*	88	8	12	135	15.3	1.9	1.3
Subclover silage*	45	9	16	83	18.4	2.0	1.2
Maize greenchop*	35	10	6	45	12.9	1.3	2.1
Wheat feed**	90	13	-	200	22.2	1.7	-
Lucerne hay**	90	8.5	-	300	33.3	3.9	-
Lupins**	90	-	32	450	50.0	-	1.6
Urea lick blocks**	100	-	40	850	85.0	-	2.1
MBM	**100**	**12.9**	**53.2**	**600**	**60**	**4.7**	**1.1**
Source	DM (%)	Metabolisable energy (MJ kg DM^{-1})	CP (% DM)	$ t^{-1}	$cent kg DM^{-1}	$cent MJ^{-1}	$ kg CP^{-1}
PPB	100	16.8	62.9	62	6.2	0.4	0.1
PPB	100	16.8	62.9	100	10.0	0.6	0.2
PPB	100	16.8	62.9	200	20.0	1.2	0.3
PPB	100	16.8	62.9	400	40.0	2.4	0.6
PPB	**100**	**16.8**	**62.9**	**600**	**60.0**	**3.6**	**1.0**
PPB	100	16.8	62.9	1000	100.0	6.0	1.6

From:*[64], **[65]

The comparison in Table 8 shows the potential value of PPB biomass, however the real world value and marketability is still being investigated. Research to evaluate the applicability of PPB biomass as organic fertiliser as well as the nutritional value characterization is ongoing (RnD4Profit Waste to Revenue: Novel Fertilisers and Feeds. APL; No. 2014/534.05). The application of PPB biomass as feed additive depends on local legislation and has to be determined for every case.

Figure 8 shows a comparison of the value of 1.0 m^3 of red meat processing wastewater in the case of water, N, P and COD (as methane) recovery compared to the value of PPB produced from 1.0 m^3 of wastewater (WW). If PPB biomass is considered as an analogue to MBM and values of \$400-600 t^{-1} are achievable, this technology would increase the value of meat processing wastewater by up to 450%, compared to conventional technologies. The values of water (\$0.41 m^{-3}), N (\$0.19 kg^{-1}), P (\$1.17 kg^{-1}) and methane (\$10 GJ^{-1}) used in this analysis are adapted from a combination of industry knowledge and literature [66]. The value of the PPB biomass does not include the revenue for potential water recovery.

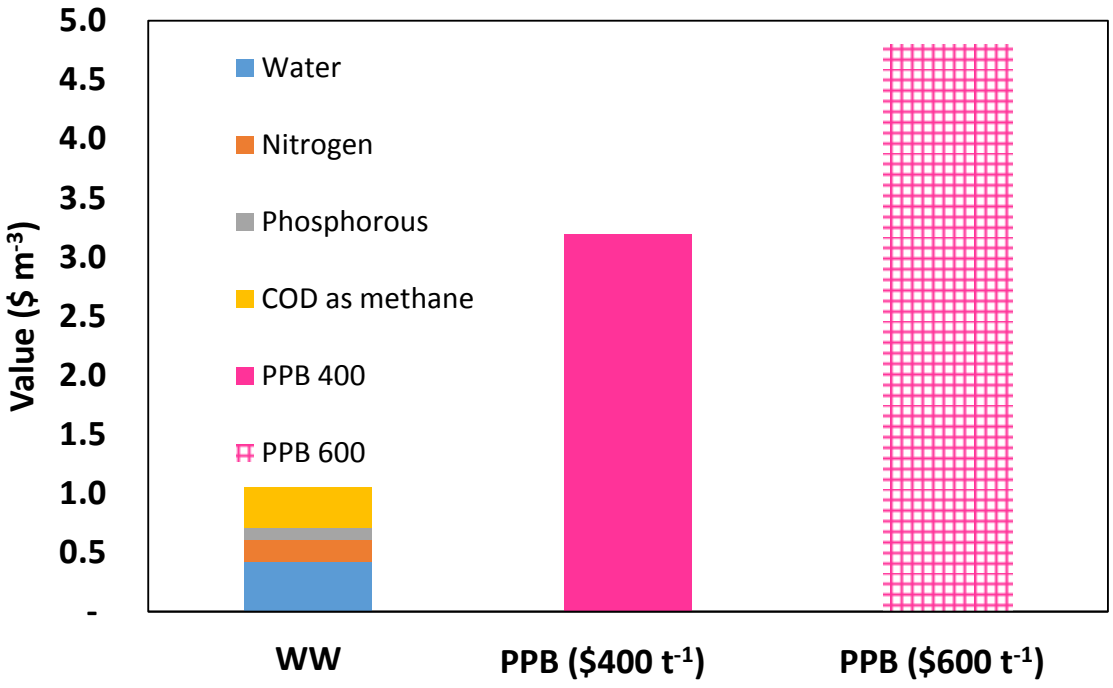

Figure 8: Potential value of 1.0 m^3 of red meat processing wastewater when recovering all resources (WW), selling the biomass at \$400 t^{-1} and \$600 t^{-1}.

Application of PPB for Red Meat Processing Wastewater Treatment

The use of PPB for red meat processing wastewater has not been reported in available literature. Therefore this report extrapolates experiences in application of PPB to domestic wastewater and other industrial wastewaters to assess the potential for slaughterhouse applications and the key technical risks.

Research on domestic wastewater revealed a number of process and design parameters relevant to red meat processing applications which will be used for the Case Study calculations presented in Sections 0, 0 and 0. The recommended reactor configuration is an IR illuminated photo anaerobic membrane bioreactor (PAnMBR) with a hydraulic retention time of 1 day and a solids retention time of 2 days.

Work in domestic wastewater has demonstrated COD, TN and TP removal efficiencies in the PAnMBR of over 95%, 84% and 93% at an organic loading rate up to 3 kgCOD $m^{-3}d^{-1}$. Effluent COD is generally less than 200 mg L^{-1} and is therefore similar to the best performing lagoon based processes. Red meat processing wastewater contains high amounts of particulate organics with a relatively low soluble fraction (~20%). While PPB can grow with various organic compounds they are generally limited to low molecular weight and soluble components [50]. However, particulate organics in slaughterhouse wastewater are known to be highly degradable by anaerobic bacteria and therefore the ability of PPB to utilise particulate COD in these streams is considered low risk. When applied to sardine processing wastewater with up to 60 gCOD L^{-1} and excessive mineral solids (up to 201 g L^{-1} of total solids), PPB were able to remove >70% of COD [40]. This indicates that PPB can be applied effectively to waste streams with high solids.

In addition to a high fraction of particulate COD, the high FOG content (1000 to 3000 mg L^{-1}) of slaughterhouse wastewater may present a challenge for PPB. FOG is known to cause problems with sludge settleability, and while the membrane in the PAnMBR would limit the loss of PPB, poor settleability would make harvesting the biomass more challenging. High FOG concentrations have been shown to increase the risk of microbial inhibition in some applications (e.g. anaerobic digesters), however FOG is readily degradable and may be metabolized, therefore it is not clear if the high FOG content would cause similar problems with PPB processes. At this stage FOG is flagged as an area for future investigation.

Nutrient availability is another factor that requires consideration. PPB simultaneously remove COD, N and P whereby the removal efficiency of each component depends on the ratios. Ideal ratios for complete removal of COD, N and P are around 100:6.0:1.0, this is based on a PPB population enriched on domestic wastewater and dominated by *Rhodobacter spp*. The average characteristics of slaughterhouse wastewater after primary treatment/solids removal (as summarized in Table 1 and Table 2) show that typical COD:N:P ratios of Australian slaughterhouse wastewater are approximately 100:2.4:0.4 – suggesting an excess of COD (and limitation of N and P). We expect a different PPB community profile for red meat processing wastewater and this will likely result in different ideal COD:N:P ratios, however this is an area that requires further research.

Assuming simultaneous COD, N and P removal in the PAnMBR, a PPB process would be a single-step treatment process for slaughterhouse wastewater. Due to near complete removal of TN and TP and biomass retention with a membrane, the effluent is expected to reach discharge limits without further post-treatment. Aerobic polishing will be unnecessary and the sludge stream is expected to have value-add applications as organic fertiliser or as a protein-rich feed additive.

In a slaughterhouse context, a PPB process should be positioned after primary treatment/solids removal (screening, precipitation and/or DAF). Figure 9 gives an overview of typical wastewater treatment trains and the recommended positioning of PPB, although this may be revised as the technology develops. More detailed descriptions of existing wastewater treatment practices are presented in Section 5.5.

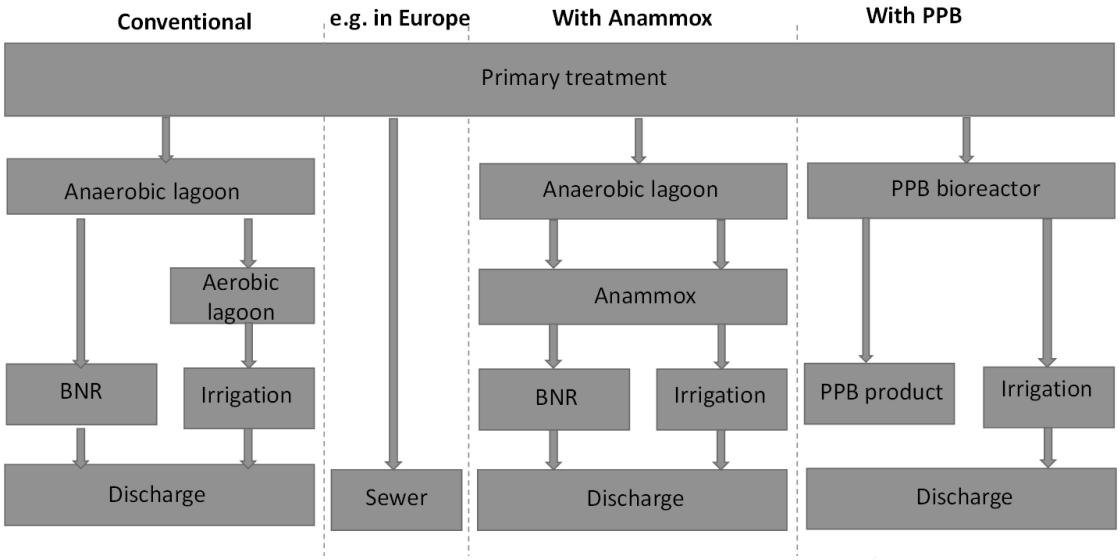

Figure 9: Comparison of treatment option for red meat wastewater and principal location of treatment steps.

Comparison to Competing Technologies Focused on Waste-to-Protein

Algae and cyanobacteria are competing technologies which are based on phototrophic organisms that target protein recovery during wastewater treatment. In this report, the term microalgae will be used to collectively describe both; microalgae and cyanobacteria.

Traditionally, microalgae for wastewater treatment was applied as a final polishing step for secondary or tertiary effluent [67]. However, more recent advances target application to primary wastewater with a focus on simultaneous C, N and P removal for recovery [68].

Waste stabilisation ponds (WSP) as described by Oswald, Gotaas [69] are the most widely used phototrophic treatment technology. These systems include mixed cultures of nitrifying, denitrifying bacteria, algae, cyanobacteria and protozoa whereby the bacteria utilise the oxygen produced by algae for nitrification and COD oxidation. Nitrate produced during nitrification is transformed to nitrogen gas by denitrifying organisms. WSPs have low capital and operational costs but are almost exclusively applied in rural areas due to very large footprints. The biomass productivity is rather low and the biomass is a mixture of several microorganisms rather than algae only, which lowers the potential value of the biomass.

Open raceway ponds or high rate algal ponds (HRAP) have higher productivity and are mainly used for commercial biomass growth for biofuels and health products. However, HRAP are also applied for wastewater treatment. HRAP are relatively cheap to operate but have low biomass productivities and require large surface areas.

Alternatively, closed photo-bioreactors (PBRs) have been applied for microalgae cultivation with a focus on bioenergy rather than wastewater treatment. Closed PBRs have a smaller footprint and higher biomass productivity but have high capital and high operational costs.

Due to high costs closed PBRs are predominantly used for the growth of axenic monocultures to produce high-value products. This technology is usually not targeted for wastewater treatment.

Table 9 gives an overview of the most common large-scale phototroph cultivation systems such as: WSPs, HRAPs and tubular photo-bioreactors as well as the PAnMBR with PPB.

Table 19: Comparison of the process features of different algal and cyanobacteria technologies and PPB in a PAnMBR.

		Waste stabilization pond (WSP)	High rate algal pond (HRAP)	Photo-bioreactor (tubular)	PAnMBR with PPB
Volumetric biomass productivity	g L^{-1} d^{-1}		[2] 0.035	[2] 0.56	1-3
Hydraulic retention time	d	[2] 10	[2] 10	[2] 10	0.5-1
Footprint	m^{-2} m^{-3}	Large	Large	Small	small
Illuminated surface/volume ratio	m^{-2} m^{-3}	[2] 3.3	[2] 3.3	[2] 99	99
COD removal	%		[1] 76 (65 – 87)		90 (85-95)
TN removal	%		[3] 67.1 (36–87.2)	[3] 78.5 (68-89.7)	95 (90-99)
TP removal	%		[3] 52.1 (32–72.9)	[3] 93.2 (85-99)	95 (90-99)
Energy demand	-	low	low	high	medium
Illumination intensity	W m^{-2}	<100 (sunlight)	<100 (sunlight)	<100	5-20
Mixing energy	kJ m^{-3}		[3] 3.2 – 9.6*	[3] 6300 – 13000**	540
Operational costs	-	low	low	high	medium
Capital costs	-	low	low	high	medium
Other process issues		Very large footprint, water evaporation, high harvesting costs	Very large footprint, water evaporation, high harvesting costs	Mainly used for axenic cultures, high value chemical production	Only lab-scale experience

Data in brackets are min and max values.

*paddle wheel, ** aeration, *** mechanical mixing, data from [1][70], [2] [71], [3] [72]

23.0 Potential Value of Algal Biomass

Microalgae have been intensively studied for biofuel production and the main barrier currently limiting commercialisation is the high production cost. Biofuel derived from algal biomass has to compete with crude oil prices (e.g. US$1.13 kg^{-1}). For algae containing 40% oil content, the production cost has to achieve US$0.45 kg^{-1} to be competitive.

While the value of algae can be improved by selling the remaining fraction (after oil extraction) as protein rich feedstock the value is still not competitive with current oil prices [73].

However, algal biomass contains several other components such as β-carotene, astaxanthin, docosahexaenoic acid, eicosahexaenoic acid, phycobilin pigments and algal extracts for use in cosmetics. Microalgae are also increasingly playing a role in cosmeceuticals, nutraceuticals and functional foods [73]. The cost-benefit of these high value products can be highly variable. For example, *D.salina* was the first algae commercialised with a value between US$ 300-1,500 kg^{-1} and this was mainly due to its high content of natural β-carotene. The second commercialised carotenoid from algae was astaxanthin from the freshwater green alga *H. pluvialis* [74]. However, cultures producing high value chemicals are grown in closed PBR as monocultures with specific substrates. In most cases wastewater cannot be sterilised and the wastewater characteristics are not consistent enough for these applications.

Similar to PPB, algal and microalgal biomass can be marketed as feed or a feed additive rich in protein, fats and vitamins A, B, C, D and E. Decades of trials established the positive aspects of small amounts of microalgae as a feed additive (almost exclusively of the genera *Chlorella*, *Scenedesmus* and *Spirulina)*. Algae are now used successfully as a feed additive for poultry and aquaculture. Pet food is another emerging market [75].

While algae are suitable for animal consumption, they are not suited to human consumption without purification. Humans lack the cellulase enzyme and cannot degrade algal cell walls. In this context, nucleic acid safety is a concern in bacterial single cell protein. Intake of a diet high in nucleic acid content leads to the production of uric acid from nucleic acid degradation [76]. Algae have lower nucleic acid than bacteria. However, PPB are different due to the phototrophic metabolism and the nutritional values including the nucleic acid content has to be determined.

A large number of nutritional and toxicological evaluations demonstrated the suitability of algae biomass as a valuable feed supplement or substitute for conventional protein sources (soybean meal, fish meal, rice bran, etc.) [77]. A comparison of the nutritional value of algal biomass, MBM and PPB is shown in Table 20.

Table 20: Comparison of energy (MJ) and crude protein (CP) content of algal biomass with MBM and PPB.

	MBM		Algae		PPB AVG	
	% DM	MJ kg^{-1}	% DM	MJ kg^{-1}	% DM	MJ kg^{-1}
Crude protein	50	8.4	50	8.4	62.9	10.5
Crude fat	10	3.8	7.5	2.8	5.2	2.0
Soluble carbohydrates	-		9	1.5	23.2	3.9
Crude fiber	-		3	-	-	-
Ash	34		3	-	-	-
Total	-	12.1	-	12.7	-	16.3

24.0 Drawbacks of Microalgae Treatment Systems

Claimed advantages of phototrophic consortia over conventional wastewater treatment are energy savings due to the oxygenation potential in a mixotrophic consortium of bacteria and algae, the potential nutrient recovery and biofuel production.

However, producing biofuels from algae remains uneconomic, and significant further research is needed [78]. Challenges of algae technologies include light and CO_2 supply, pH adjustment, water evaporation, unstable consortia, grazing, high thickening costs, and potentially very large footprints [79]. Currently the most cost effective photo-bioreactor is the open raceway pond [80] with a huge footprint due to shallow water for light penetration and a 10 day HRT [71]. Closed photo-bioreactors have a smaller footprint but current investment costs significantly exceed the price for economical production of energy products and render wastewater treatment by algae economically unfeasible [81].

Similar to reports about algae, cyanobacteria have been applied for sec [82] and tertiary sewage treatment [83] but also for a wide range of industrial wastewaters [84]. Most of the studies applied axenic cultures in batch tests. Usually cyanobacteria are part of a microalgae consortium.

Cyanobacteria were reported to be able to outcompete microalgae mostly due to lower illumination intensity requirements (e.g. 6- 25Wm^{-2} [85]) and higher affinity to N and P [86]) although the general growth rates are slower compared to most microalgae [87]. Some species are photoheterotrophic but the majority rely on CO_2 addition and reported HRTs were several days [87]. In fact, the drawbacks listed for algae are valid for cyanobacteria as mediator as well. Lower light intensities and consequently less heat evaporation are in favour of cyanobacteria. However, the major problem with cyanobacteria is the potential production of more than 80 microtoxins produced by different cyanobacteria [88]. Among the freshwater species only a small number is toxic but blooms are mostly formed by toxic and non-toxic strains whereby the mechanisms and selection factors are unclear [88]. The occurrence of microtoxins in wastewater has been reported [89] and phytoplankton bloom containing elevated levels of microcystin producing *microcystis aeruginosa* are common in wastewater treatment plants [90]. This is considered to be a major reason against cyanobacteria use for wastewater treatment.

In general, the microbial population shifts and the process conditions have to be closely monitored to ensure dominance of microalgae over other microorganisms. Saline conditions usually reduce the risk of contamination but this is not applicable for agricultural wastewater treatment. A variety of species will also reduce the potential value due to composition changes.

25.0 Appendix C: Case Studies to Assess the Economics of Different Treatment Options

This section includes a basic assessment of potential wastewater treatment configurations for the Australian red meat processing industry. These options include:

- Covered anaerobic lagoon + BNR
- Covered anaerobic lagoon + BNR with WAS recycle to CAL
- Covered anaerobic lagoon + anammox
- AnMBR + anammox
- High Rate SBR + AD + anammox
- PAnMBR with PPB
- PAnMBR + AD + anammox
- CAL + PAnMBR
- Algae and cyanobacteria

The costing information in this analysis is adopted from A.ENV.0162. This report evaluated nitrogen removal technologies, but did not originally consider PPB, algae or cyanobacteria. The comparisons are based on treatment processes designed to produce wastewater with less than 50mg L^{-1} total N and therefore suitable for irrigation. The case studies are not designed to test the removal limits of the technologies. The comparison that follows is based on order of magnitude estimates and is not intended as a detailed feasibility analysis; it is intended as an indication of the relative contributions of the organic removal and nitrogen removal steps to capital and operating costs.

Capital costs are generally estimated using plant/vessel size and a linear cost basis. However, there are likely some economies of scale, particularly for larger process vessels. Final vessel cost will be dependent on final design, construction material selection/availability (e.g. concrete, stainless steel, mild steel, glass panelling) and local suppliers or contractors.

Basis used in Case Study Analyses

The case study used to examine treatment technologies is based on treatment of the combined wastewater for a processing plant after primary solids removal and before anaerobic treatment, the cost associated with the anaerobic treatment and the value of biogas recovered is included in the assessment. The analysis is based on a facility processing 600 head of cattle per day, with total effluent flow of 1.7 ML d^{-1}. Inputs are based on nutrient and organic contaminant production (per THSCW) as reported in recent MLA and AMPC projects (A.ENV.0131 and A.ENV.0151).

Each treatment technology has been developed to achieve a total nitrogen discharge of approximately 50 mg L^{-1} this corresponds to approximately 80% total N removal.

Table 21: Wastewater flow, concentration and load for case study the different process alternatives

	Concentration		Load	
Production level			600	head d^{-1}
Wastewater volume			1730	kL d^{-1}
COD	10,000	mg L^{-1}	17,300	kg d^{-1}
Solids	3,480	mg L^{-1}	10,000	kg d^{-1}
Nitrogen	250	mgN L^{-1}	936	kg d^{-1}
Phosphorous	50	mgP L^{-1}	144	kg d^{-1}

Phosphorus (P) recovery using struvite crystallisation ($NH_4MgPO_4 \cdot 6H_2O$) is an emerging technology option that may be integrated into the treatment process where P removal is required. The specific costs around P recovery are not included. However if applicable, the process flowsheets demonstrate where the P recovery unit could be placed in each process.

Covered Anaerobic Lagoon with Nitrification/Denitrification

Treatment using an anaerobic lagoon (CAL) followed by aerated lagoons or SBRs for nitrification and denitrification (BNR) is currently one of the most commonly applied process configuration for treatment of slaughterhouse wastewater. Therefore, treatment using a CAL and BNR will represent the default treatment option (Figure 10). The specific process assessed in this report was described in ENV.044. In this process, approximately 20% of raw wastewater is diverted past the CAL to provide a carbon source for the denitrification step, pre-fermentation can be used to produce VFA and assist in P removal. Alternatively, an external carbon source such as methanol could be supplied; but this would result in significant chemical consumption costs and is not considered in this analysis. The nitrification/denitrification steps will produce waste sludge that requires treatment and disposal; this could be done in the CAL, in a separate in-vessel digester or off-site.

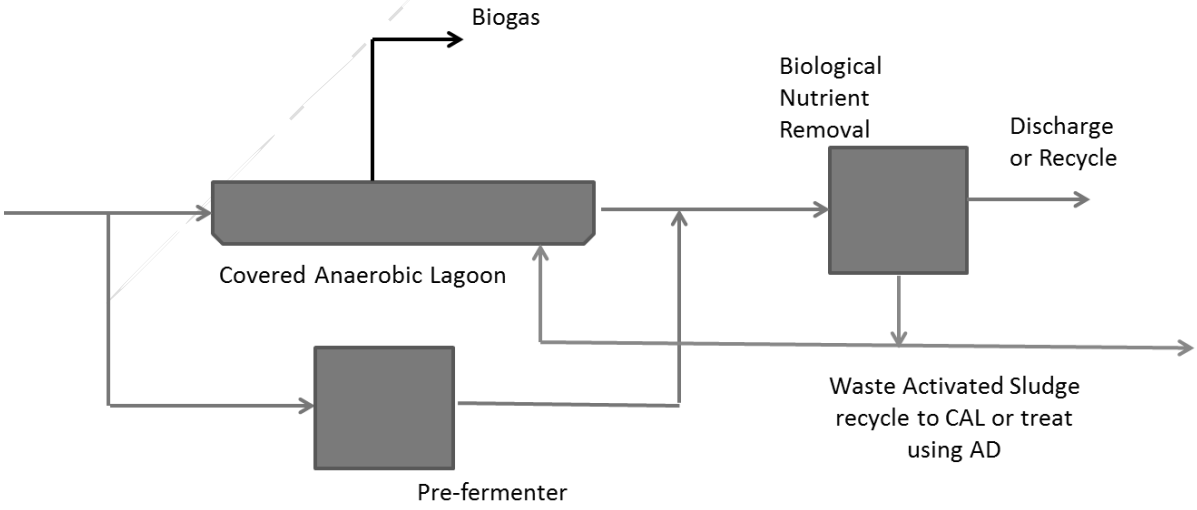

Figure 10: Process flowsheet representing a covered anaerobic lagoon followed by nitrification/denitrification in an SBR. The process is similar to that presented in ENV.044.

The covered anaerobic lagoon is sized based on a hydraulic retention time of 30 days and is largely oversized. The anaerobic biodegradability of the organic material is 90% (based on findings from MLA/AMPC projects A.ENV.0131 and A.ENV.0151). CAL efficiency is set to 80% of degradable COD. Where the WAS is recycled through the CAL, the anaerobic biodegradability of the WAS is estimated at 40%.

The nitrification/denitrification is based on the BNR pilot plant designed and operated in MLA project ENV.044. The SBR operated at a HRT of 2 days. Results from ENV.044 demonstrate this is sufficient for COD and N removal at 90%.

Sludge production was calculated based on a yield of 0.4 kgSS $kgCOD^{-1}$ feed, which is high for BNR processes. The composition of activated sludge produced in the BNR was 0.08 kgN $kgCOD^{-1}$. Energy demand was calculated as 4.6 kWh per kgN (removed as N_2) and 1 kWh $kgCOD^{-1}$ that was oxidised. For the CAL, capital costing was estimated at \$10 per m^3 for excavation, \$20 per m^2 for pond lining and \$25 per m^2 for the pond cover (*personal communication, Stephan Tait – Pork CRC Bioenergy Support Programme*). For the BNR, capital costing was estimated at \$800 per m^3 tank volume (*personal communication, Prof Jurg Keller, UQ*).

Ancillary costs include foundations, pumps, piping and instruments and were correlated with the capital cost of the process vessels. Operating costs are estimated based on current pricing, including electricity at \$0.1 kWh^{-1}, personnel at \$80,000 per full time equivalent, maintenance of 2-4% of initial capital per annum. Value recovery is based on a gas value of \$10 GJ^{-1}.

A summary of capital costs for a CAL and BNR process is shown in Table 22, a summary of operating costs for a CAL and BNR process is shown in Table 23. The operating expenses shown in Table 23 do not consider the costs of sludge disposal which may be in the range of \$30-\$100 t^{-1}. Processors may operate the SBR to minimise sludge production, if the sludge yield is reduced to 0.2 kgSS $kgCOD^{-1}$ added the aeration costs would increase by \$75,000 per year to oxidise additional COD and maintain the COD and N removal rates.

Table 22: Summary of capital costs for a covered anaerobic lagoon followed by nitrification and denitrification

	Basis	Estimated Capital
Covered anaerobic lagoon	6912 m^2 area and 6 m depth	\$726,000
Cogeneration unit	562 kW @ \$1,500 kW^{-1}	\$844,000
BNR	3460 m^3 @ \$800 m^{-3}	\$2,765,000
Installation and ancillaries		\$344,000
Engineering costs	10% of capital	\$468,000
Total estimated capital		\$5,147,000

Table 23: Summary of operating costs for covered anaerobic lagoon followed by nitrification and denitrification

	Basis	Estimated Expenditure
Operator support	0.35 FTE at $80,000	$28,000
Vessel and pipe maintenance	2-4% capital	$119,000
CAL energy demand	0.01 kWh $m^{-3}d^{-1}$	$15,000
BNR energy demand	4.6 kWh kgN^{-1} and 1kWh $kgCOD^{-1}$	$58,000
Electricity generation from co-gen	$0.1 kWh^{-1}	-$493,000
Renewable energy credits	$0.034 kWh^{-1}	-$172,000
Total estimated operating		-$445,000

Initial calculations were based on transport of waste sludge offsite for processing (costs not considered), additional calculations were conducted where the waste sludge was recycled into the CAL for treatment. Recycling the waste sludge to the CAL had little impact on capital cost due to the relatively low volume. Interestingly, recycling the waste sludge into the CAL also had little impact on the overall operating costs as the increased methane production from sludge degradation offset increased aeration demand from recycling the sludge COD into the BNR.

Covered Anaerobic Lagoon Coupled to Anaerobic Ammonium Removal

Anammox is an emerging low-energy technology for nitrogen removal. If applied as an add-on to existing slaughterhouse applications, the recommended process configuration would be a covered anaerobic lagoon (CAL) to remove organic contaminants, followed by anammox in an SBR style reactor. A simplified process flowsheet is presented in Figure 11.

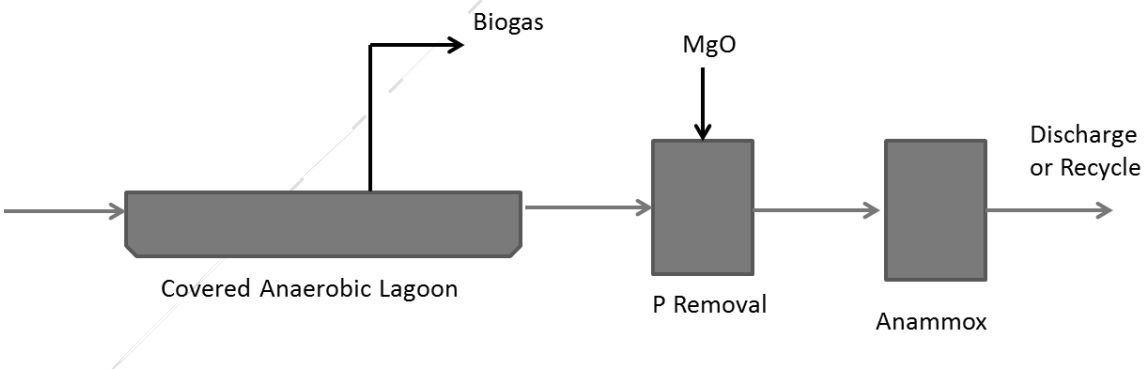

Figure 11: Process flow sheet representing covered anaerobic lagoon followed by anaerobic ammonium removal; Phosphorus removal is optional and is not included in cost calculations.

Design and costing of the covered anaerobic lagoon is similar to Section 0. The anaerobic biodegradability of the raw slaughterhouse wastewater is 90%. CAL efficiency is set to 80% of degradable COD. Therefore 72% of COD entering the pond is converted to biogas. The CAL would be approximately 20% larger as raw wastewater is no longer diverted to the nitrogen removal step. The anammox process is based on an SBR with a nitrogen loading rate of 0.7 kg m^{-3} d^{-1}. The energy demand for N removal was 1.2 kWh kgN^{-1} removed.

The effluent quality was set at 25mg L^{-1} NH_4^+, 10 mg L^{-1} NO_2 and 20 mg L^{-1} NO_3 (10% of NH_4^+ removed), this results in an effluent concentration of 55 mg L^{-1} total nitrogen. In addition to N removal, the anammox reactor was assumed to oxidise 20% of the COD feed. Energy demand for the COD removal was calculated at 1 kWh $kgCOD_{removed}^{-1}$. For the anammox reactor, capital costing was estimated at $800 per m^3 tank volume.

Ancillary costs include foundations, pumps, piping and instruments and were correlated with the capital cost of the process vessels. Operating costs are estimated based on current pricing, including electricity at $0.1 kWh^{-1}, personnel at $80,000 per full time equivalent, maintenance of 2-4% of initial capital per annum. Value recovery is based on cogeneration efficiency of 0.35 and $0.1 kWh^{-1}, this corresponds to a gas value of $10 GJ^{-1}.

A summary of capital costs for a CAL and anammox process is shown in Table 24, a summary of operating costs for a CAL and anammox process is shown in Table 25.

Table 24: Summary of capital costs for anaerobic ammonium removal coupled to covered anaerobic lagoon

	Basis	Estimated Capital
Covered anaerobic lagoon	8640 m^2 area and 6 m depth	$907,000
Cogeneration unit	703 kW @ $1,500 kW^{-1}	$1,055,000
Anammox Reactor	555 m^3 @ $800 m^{-3}	$444,000
Installation and ancillaries		$186,000
Engineering costs	10% of capital	$259,000
Total estimated capital		$2,851000

Table 25: Summary of operating costs for anaerobic ammonium removal coupled to covered anaerobic lagoon

	Basis	Estimated Expenditure
Operator support	0.35 FTE at $80,000	$28,000
Vessel and pipe maintenance	2-4% capital	$83,000
CAL energy demand	0.01 kWh $m^{-3}d^{-1}$	$19,000
Anammox energy demand	1.2 kWh $kgN^{-1}_{removed}$	$28,000
Electricity generation from co-gen	$0.1 kWh^{-1}	-$616,000
Renewable energy credits	$0.034 kWh^{-1}	-$216,000
Total estimated operating		-$674,000

Anaerobic Membrane Bioreactor Coupled to Anaerobic Ammonium Removal

Anaerobic membrane bioreactors (AnMBR) are emerging as an alternative technology to CALs for treatment of organic materials. AnMBRs are a high rate anaerobic technology that utilise a membrane to retain biomass and residual substrate within the reactor. The membrane separates the hydraulic retention time and the solids retention time, as a result AnMBRs are able to operation at very short hydraulic retention times compared to CALs.

In this process configuration an AnMBR is used to remove organic contaminants, followed by anammox in an SBR style reactor. A simplified process flowsheet is presented in Figure 12.

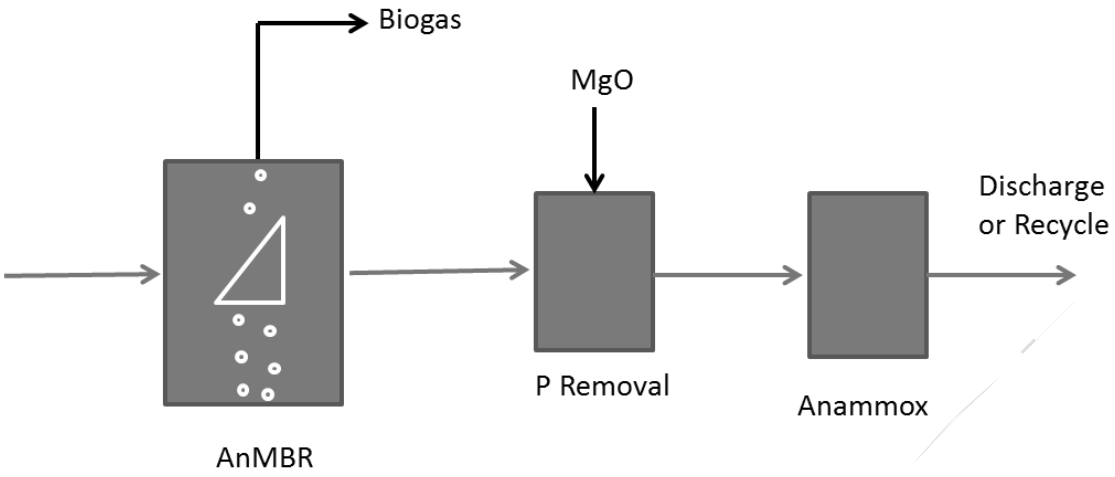

Figure 12: Process flow sheet representing anaerobic membrane bioreactor followed by anaerobic ammonium removal; Phosphorus removal is optional and is not included in cost calculations.

The AnMBR is designed based on a hydraulic retention time of 2.0 days and an OLR of 5.0 kg $m^{-3}d^{-1}$. The required membrane surface area is based on 10 L $m^{-2}h^{-1}$. The anaerobic biodegradability of the organic material is 90% (based on findings from MLA/AMPC projects A.ENV.0131 and A.ENV.0151). AnMBR efficiency will be higher than a CAL and is set to 95% of degradable COD (based on findings from MLA/AMPC projects A.ENV.0133 and A.ENV.0149). Therefore 86% of COD entering the AnMBR is converted to biogas. The increased efficiency in the AnMBR also results in a greater conversion of organic N and a higher concentration of N transferred to the anammox reactor. For the AnMBR, capital costing was again estimated at $1,000 per m^3 tank volume including an allowance for membranes.

The anammox process was designed using the guidelines discussed in Section 0. The anammox was sized using a loading rate of 0.7 kgN m^{-3} d^{-1}. Energy demand was based on 20% of feed COD being oxidised (1kWh $kgCOD^{-1}$) and 1.2 kWh kgN^{-1} removed. Again, the effluent quality was set at 25mg L^{-1} NH_4^+, 10 mg L^{-1} NO_2 and 22.5 mg L^{-1} NO_3 (10% of NH_4^+ removed), this results in an effluent concentration of 58 mg L^{-1} total nitrogen. For the SBR, capital costing was again estimated at $800 per m^3 tank volume.

Again, ancillary costs include foundations, pumps, piping and instruments and were correlated with the capital cost of the process vessels. Operating costs are estimated based on current pricing, including electricity at $0.1 kWh^{-1}, personnel at $80,000 per full time equivalent, maintenance of 2-4% of initial capital per annum. Value recovery is based on cogeneration efficiency of 0.35 and $0.1 kWh^{-1}, this corresponds to a gas value of $10 GJ^{-1}.
A summary of capital costs for an AnMBR and anammox process is shown in Table 26, a summary of operating costs for an AnMBR and anammox process is shown in Table 27.

Table 26: Summary of capital costs for anaerobic ammonium removal coupled to covered anaerobic lagoon

	Basis	Estimated Capital
AnMBR	3460 m^3 @ \$1000 m^{-3}	\$3,460,000
Membranes	7208 m^2 @ \$60 m^{-2}	432,480
Cogeneration unit	835 kW @ \$1,500 kW^{-1}	\$1,252,000
Anammox Reactor	617 m^3@ \$800 m^{-3}	\$494,000
Installation and ancillaries		\$212,501
Engineering costs	10% of capital	\$572,460
Total estimated capital		\$6,423,441

Table 27: Summary of operating costs for anaerobic ammonium removal coupled to covered anaerobic lagoon

	Basis	Estimated Expenditure
Operator support	0.35 FTE at \$80,000	\$28,000
Vessel and pipe maintenance	2-4% capital	\$128,460
AnMBR energy demand	0.4 kWh $m^{-3}year^{-1}$	\$50,516
Anammox energy demand	1.2 kWh kgN^{-1} and 1kWh $kgCOD^{-1}$	\$23,000
Electricity generation from co-gen	\$0.1 kWh^{-1}	-\$731,000
Renewable energy credits	\$0.034 kWh^{-1}	-\$256,000
Total estimated operating		-\$757,024

High Rate Aerobic Treatment Coupled to Anaerobic Digestion (A.ENV.0150)

High rate aerobic treatment coupled to anaerobic digestion is a technology option designed to treat slaughterhouse wastewater and is currently under development in AMPC/MLA project A.ENV.0150. A simplified process flowsheet is presented in Figure 13 and mainly consists of a sequencing batch reactor (SBR) for carbon removal and partial nutrient removal, an anaerobic digester for solids stabilization, a struvite crystallizer for nutrient recovery and an anammox reactor for effluent polishing to achieve a discharge concentration of approximately 50 mg N L^{-1}.

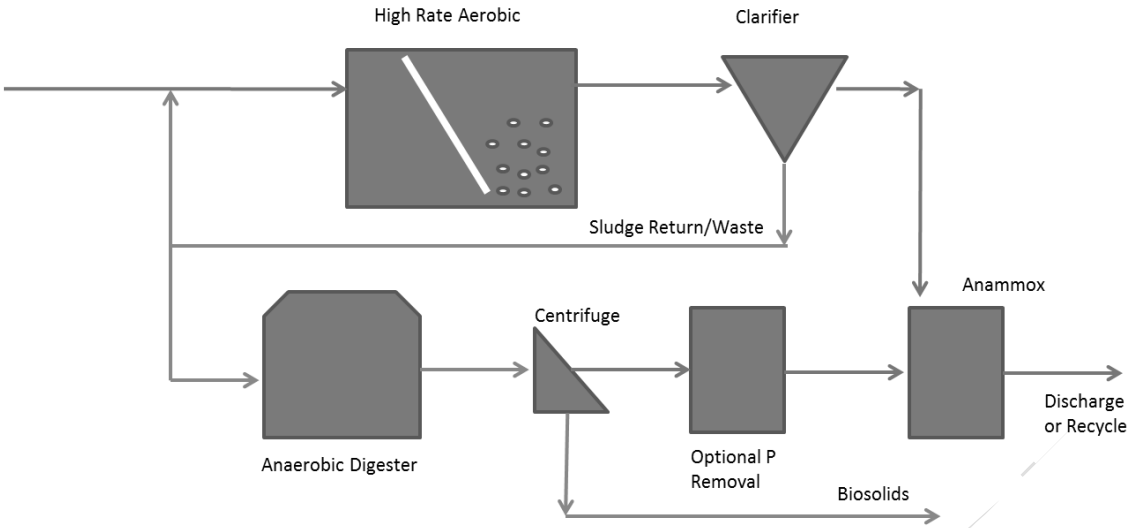

Figure 13: High rate aerobic wastewater treatment and anaerobic digestion with anammox integrated for treatment of side stream AD centrate (A.ENV.0150)

The high rate SBR is not a nitrification/denitrification process; the nitrogen is removed through biomass growth only and does not leave the process as N_2 gas. The high-rate SBR is designed to have a hydraulic retention time (HRT) of 0.5 days and sludge retention time (SRT) of 2 days, this is significantly shorter than a conventional SBR for nutrient removal and aims to convert organic matter (measured as COD) into biomass, instead of oxidising it. This will reduce aeration requirements (resulting in lower energy demands) while achieving partial nutrient capture in the biomass growth (e.g. approx. 60% total nitrogen capture and 70% total phosphorus capture). The biomass generated from the SBR is thickened to 4% solids and treated in a mesophilic anaerobic digester (37°C and 12 day HRT), where approximately 60% of the biomass is converted to biogas. The stabilized solids stream is dewatered by a centrifuge, with the solids cake being transported for land application, the cost of transport and land application is not included in this analysis.

The effluent streams from the high rate SBR and the digester are combined for further treatment using anaerobic ammonium removal. The anammox process is designed according to Section 0.

Again, ancillary costs include foundations, pumps, piping and instruments and were correlated with the capital cost of the process vessels. Operating costs are estimated based on current pricing, including electricity at \$0.1 kWh^{-1}, personnel at \$80,000 per full time equivalent, maintenance of 2-4% of initial capital per annum. Value recovery is based on cogeneration efficiency of 0.35 and \$0.1 kWh^{-1}, this corresponds to a gas value of \$10 GJ^{-1}.

A summary of capital costs for a high rate aerobic nitrogen removal followed by an in-vessel anaerobic digester is shown in

Table 28, a summary of operating costs is shown in

Table 29.

Table 28: Summary of capital costs for high rate nitrogen removal coupled to in vessel anaerobic digestion

	Basis	Estimated Capital
Anaerobic digester	2200 m^3@ \$800 m^{-3}	\$2,228,000
Cogeneration unit	562 kW @ \$1,500 kW^{-1}	\$844,000
SBR	864 m^3 @ \$800 m^{-3}	\$691,000
Anammox reactor	358 m^3@ \$800 m^{-3}	\$286,000
Installation and ancillaries		\$293,000
Engineering costs	10% of capital	\$434,000
Total estimated capital		\$4,776,000

Table 29: Summary of operating costs for high rate nitrogen removal coupled to in vessel anaerobic digestion

	Basis	Estimated Expenditure
Operator support	0.35 FTE at \$80,000	\$28,000
Vessel and pipe maintenance	2-4% capital	\$102,000
Digester mixing energy	0.1 kWh $m^{-3}d^{-1}$	\$10,000
Dewatering energy		\$60,000
SBR energy demand	1 kWh $kgCOD^{-1}$	\$56,000
Anammox energy demand	1.2 kWh kgN^{-1}	\$11,000
Electricity generation from co-gen	\$0.1 kWh^{-1}	-\$492,000
Renewable energy credits	\$0.034 kWh^{-1}	-\$172,000
Total estimated operating		-\$397,000

PAnMBR with PPB and Product Stream

The application of PPB in a PAnMBR set-up offers the potential to treat red meat wastewater in one step while achieving COD, N and P removal. We expect very low N and P effluent concentration but this depends on the overall COD bioavailability. For initial feasibility assessments we assume approximately 80% N removal (for a consistent comparison with other technologies). However, removal efficiencies up to 96% TN and 99% TP have been reported with favourable COD:N:P ratio of the wastewater.

A full-scale PAnMBR would utilise attached biomass growth on submerged illuminated surfaces. Our research showed that the solid concentration on these surfaces is >10% VS. Scraping this biomass off the illuminated surface results in thickened biomass at the bottom of the reactor. Harvesting this biomass makes extensive thickening unnecessary. However, applying thickening for further concentration is optional. Due to the composition of PPB (high protein, high P and N

content) the biomass is expected to be an excellent fertiliser or in the best case even feed additive. In this case, Figure 14 describes the PPB set-up for the red meat industry. This option includes the marketing of the PPB biomass as product.

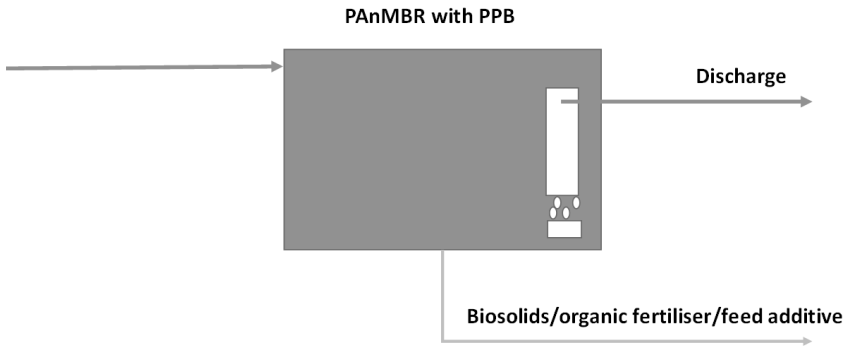

Figure 14: PPB configuration in a PAnMBR with biosolids as product stream.

The design basis for the PAnMBR process is based on a HRT of 1.0 day and a SRT of 2 days. These numbers have been used for domestic wastewater treatment and should be applicable red meat wastewater. A HRT of 1 day for slaughterhouse applications would result in an organic loading rate of 10 kgCOD m^{-3} d^{-1} which is higher than rates achieved for domestic wastewater, however the membrane prevents washout and allow for high biomass concentrations in the PAnMBR. This OLR has to be confirmed for red meat processing wastewater. The anaerobic biodegradability of the organic material is 90% with and overall COD efficiency of 95% (due to membrane). The sludge production is calculated based on 0.8 kgSS $kgCOD^{-1}$ which results in up to 13900 kg PPB biomass per day assuming good response to solid COD. The illumination energy demand is 1.0 kWh $m^{-3}_{treated}$. The mixing energy is based on 0.15 kWh m^{-3} [92] and the anaerobic membrane energy consumption is calculated with 0.4 kWh $m^{-3}_{treated}$. The energy consumption for N and P removal is included in these numbers.

The PPB capital costing is based on \$2000 m^{-3}. This includes the vessel as well as the illuminated internals and integrated harvesting system. Due to the novelty of the PPB process the cost basis is indicative and needs to be confirmed. However, a factor of 2.5 compared to BNR seems reasonable at this stage.

Ancillary costs include foundations, pumps, piping and instruments and were correlated with the capital cost of the process vessels. Operating costs are estimated based on current pricing, including electricity at \$0.1 kWh^{-1} and personnel at \$80,000 per full time equivalent. The maintenance increases from 2-4% to 4-6% of initial capital per annum due to the internals of the reactor. Value recovery is based on cogeneration efficiency of 0.35 and \$0.1 kWh^{-1}, this corresponds to a gas value of \$10 GJ^{-1}.

A summary of capital costs for the PAnMBR with PPB is shown in Table 30. The operating costs are summarized in Table 31. The operating expenses do not consider the value of PPB biomass Research is ongoing to determine the characteristics such as nutritional value (as fertiliser and animal fodder), market value and marketing strategies including early adopters.

The PAnMBR with PPB does not generate heat or electricity. However, around 3475 t year^{-1} of PPB biomass are generated during the wastewater treatment (based on 250 processing days per year). Considering the operational costs of \$313,000 year^{-1} a net price of \$90 t^{-1} would be required to off-set the operational costs. Besides studying the wastewater treatment performance of PPB, the determination of biomass characteristics grown on red meat wastewater is crucial. The value of PPB biomass has to be determined. However assuming a similar price for PPB as for MBM and other protein sources (as specified in paragraph 0) the marketing of PPB biomass as product would lead to an annual revenue of \$1,390,000 (@ \$400 t^{-1}). This would offset the operational costs and result in a payback period of 3-4 years, if higher PPB values (e.g. \$600 t^{-1}) are achieved, payback would be less than 2.5 years making PPB a very interesting, novel alternative to current, conventional and advanced technologies.

Table 30: Summary of capital costs for the PAnMBR with PPB.

	Basis	Estimated Capital
PAnMBR	1730 m^3@ \$2000 m^{-3}	\$3,460,000
Installation and ancillaries		\$275,733
Engineering costs	10% of capital	\$373,573
Total estimated capital		\$4,109,307

*unknown, assumed 2.5 times AD due to internals and IR lighting.

Table 31: Summary of operating costs for the PAnMBR with PPB

	Basis	Estimated Expenditure
Operator support	0.35 FTE at \$80,000	\$28,000
Vessel and pipe maintenance	4-6% capital*	\$186,787
PAnMBR mixing Membranes	0.15 kWh m^{-3}d^{-1} 0.4 kWh m^{-3}d^{-1}	\$9,500 \$25,300
Dewatering energy		-
Illumination energy demand	1 kWh m^{-3} **	\$64,000
Revenues organic fertiliser	To be determined, assume \$90 t^{-1}***	-\$313,587
Total estimated operating		±\$0

*increased due to complex reactor internals

** to be confirmed in pilot scale (part of CRC and APL projects).

***biomass produced is around 3475 t year^{-1}. In order to be cost neutral 1 t has to sell for \$90

PAnMBR with PPB and Anaerobic Side-stream Treatment with Nutrient Removal

An extension to the PAnMBR option is the anaerobic digestion of the PPB biomass in a side-stream set-up. One drawback of this option is the complete release of N and P as NH_4-N and PO_4-P in a concentrated side-stream. In fact, all the N and P removed from the red meat wastewater will be released during anaerobic digestion and require secondary treatment. The system configuration is shown in Figure 15.

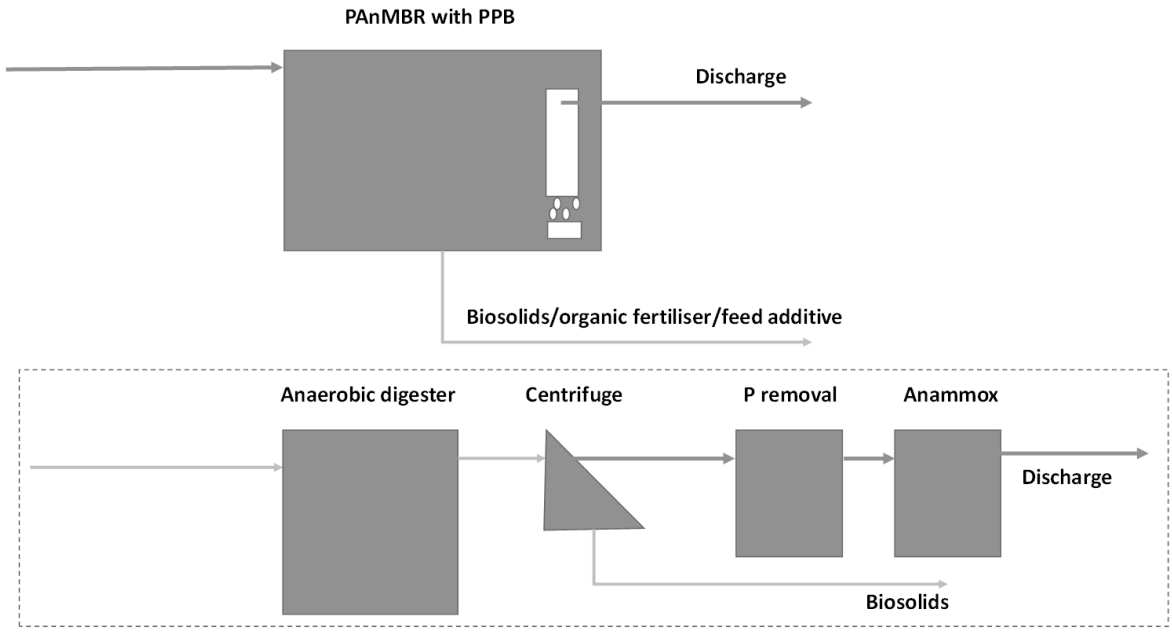

Figure 15: PAnMBR with PPB and anaerobic side stream treatment with nutrient removal.

Due to the concentrated stream it could be interesting to recover P (e.g. as struvite) and N (e.g. as ammonium sulphate). However, this will add complexity to the treatment option. Nevertheless, the amounts produced are substantial with a maximum of 936 kg N and 144 kg P (1096 t $year^{-1}$ $(NH_4)_2SO_4$ and 630 t $year^{-1}$ struvite). The value of ammonium sulphate is around \$500 t^{-1} [93] and \$400 t^{-1} struvite [94] resulting in a maximum revenue from fertiliser of \$548,000 $year^{-1}$ ($(NH_4)_2SO_4$) and \$252,000 $year^{-1}$ for struvite ($MgNH_4PO_4.6H_2O$). These numbers represent the best case and are subject to change according to fertiliser price and market development.

The energy consumption for struvite including chemicals and aeration is 5.8 kWh kgP [95], costing \$20,890 $year^{-1}$ while ammonium sulphate production consumes 25 kWh kgN^{-1} costing \$585,100 $year^{-1}$. This would leave a revenue of \$194,010 $year^{-1}$. Including the capital cost of the anammox vessel, the ammonium stripper + the acid scrubber and the P recovery vessel + the operational costs of the anammox the payback time is >5 years and not economically attractive.

The design of the anaerobic digester is based on 10% solids in the influent due to attached growth of PPB in the PAnMBR. The HRT in the mesophilic tank has to be 30 days to achieve an OLR of 3.3 kgCOD $m^{-3}d^{-1}$ and approximately 60% of the biomass is converted to biogas. The stabilized solids stream is dewatered by a centrifuge, with the solids cake being transported for land application. The cost of transport and land application is not included in this analysis.

The anammox process was designed using the guidelines discussed in Section 0. The anammox was sized using a loading rate of 0.7 kgN $m^{-3}d^{-1}$. Energy demand was based on 20% of feed COD being oxidised (1kWh $kgCOD^{-1}$) and 1.2 kWh kgN^{-1} removed.
The effluent quality was set at 25mg L^{-1} NH_4^+, 10 mg L^{-1} NO_2 and 22.5 mg L^{-1} NO_3 (10% of NH_4^+ removed), this results in an effluent concentration of 58 mg L^{-1} total nitrogen. For the SBR, capital costing was again estimated at \$800 per m^3 tank volume.

The capital and operational costs for the PAnMBR with AD side stream treatment including nutrient removal is summarized in Table 32 and Table 33. Including full side-stream treatment with AD and anammox the economics change accordingly:

Table 32: Summary of capital costs for the PAnMBR with PPB in combination with side-stream anaerobic digestion and nutrient removal.

	Basis	Estimated Capital
PAnMBR	1,730 m^3@ \$2,000 m^{-3*}	\$3,460,000
Anaerobic digester	4,671 m^3@ \$800 m^{-3}	\$3,736,800
Cogeneration unit	1,362kW @ \$1,500 kW^{-1}	\$2,043,564
Anammox	802m^3@\$800 m^{-3}	\$641,829
Installation and ancillaries		\$746,694
Engineering costs	10% of capital	\$1,062,889
Total estimated capital		\$11,691,776

Table 33: Summary of operating costs for the PAnMBR with PPB in combination with side-stream anaerobic digestion and nutrient removal.

	Basis	Estimated Expenditure
Operator support	0.35 FTE at \$80,000	\$28,000
Vessel and pipe maintenance	3-5% capital*	\$425,155
PAnMBR mixing Membranes	0.15 kWh $m^{-3}d^{-1}$ 0.4 kWh $m^{-3}d^{-1}$	\$9,500 \$25,300
Illumination energy demand	1 kWh m^{-3} **	\$64,000
Digester mixing energy	0.1 kWh $m^{-3}d^{-1}$	\$17,049
Dewatering energy	0.3kWh $kgDS^{-1}$	\$68,196
Anammox energy demand	1.2 kWh kgN^{-1}	\$24,598
Total maintenance costs		\$660,874
Electricity generation from co-gen	\$0.1 kWh^{-1} and 40% CHP eff	-\$817,425
Renewable energy credits	\$0.034 kWh^{-1}	-\$277,924
Total estimated operating		-\$434,476

* increased due to complex reactor internals of PAnMBR

PAnMBR with PPB after CAL

A third treatment configuration would be the treatment of CAL effluent by PPB (using a bypass to optimise the COD:N:P ratio for complete removal of N and P. The process configuration is shown in Figure 16. In order to achieve reasonable COD:N:P ratios in the PAnMBR around 480 m^3 d^{-1} (28% of the total flow) can be bypassed to the reactor. This results in COD:N:P ratio of 100:11:1.7. We assume that most of the Cal effluent COD is available for PPB. The PAnMBR volume is based on 1 day HRT and 2 days SRT.

The VLR would be 4.8 kgCOD $m^{-3}d^{-1}$. The biomass production based on 90% COD removal will be 1666 t $year^{-1}$ which might be sold as product with a potential yearly revenue of \$666,301 (@\$400 t^{-1}).

Table 34 summarizes the capital costs for CAL + PAnMBR and Table 35 shows the operational costs of this configuration.

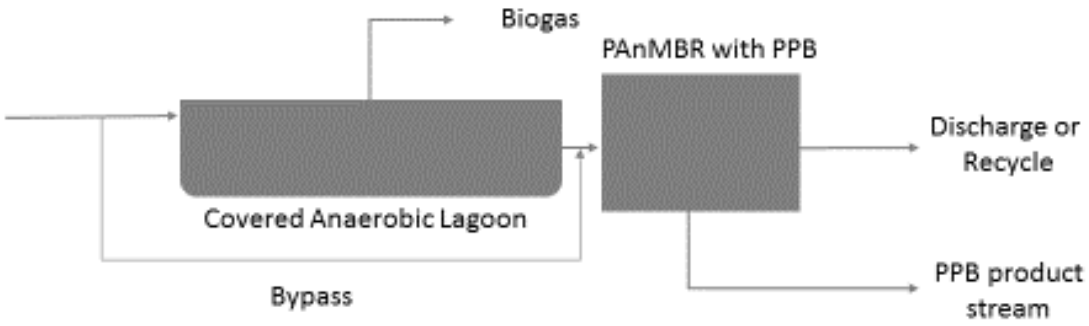

Figure 16: CAL with PAnMBR as nutrient removal step including bypass.

Table 34: Summary of capital costs for the PAnMBR with PPB in combination with side-stream anaerobic digestion and nutrient removal.

	Basis	Estimated Capital
Covered anaerobic lagoon	6221 m^2 area and 6 m depth	$653,040
Cogeneration unit	506 kW @ $1,500 kW^{-1}	$759,240
PAnMBR	1,730 m^3@ $2,000 m^{-3}*	$3,460,000
Installation and ancillaries		$346,096
Engineering costs	10% of capital	$521,874
Total estimated capital		$5,740,609

Table 35: Summary of operating costs for the PAnMBR with PPB in combination with side-stream anaerobic digestion and nutrient removal.

	Basis	Estimated Expenditure
Operator support	0.35 FTE at $80,000	$28,000
Vessel and pipe maintenance	3-5% capital*	$229,624
PAnMBR mixing Membranes	0.15 kWh $m^{-3}d^{-1}$ 0.4 kWh $m^{-3}d^{-1}$	$9,500 $25,300
Illumination energy demand	1 kWh m^{-3} **	$64,000
CAL energy	0.1 kWh $m^{-3}d^{-1}$	$13,680
Electricity generation from co-gen	$0.1 kWh^{-1} and 40% CHP eff	-$443,520
Renewable energy credits	$0.034 kWh^{-1}	-$155,520
Revenues organic fertiliser	To be determined	*
Total estimated operating		-$228,936

* assuming $400 t^{-1} and 1666 t $year^{-1}$ a yearly revenue stream of $666,301 would be generated resulting in $895,237 overall revenue.

In many cases the CAL +Cogeneration is already present which will change the capital investment. In this case only the PAnMBR has to be built which will reduce the installation costs as well as the engineering costs. The total capital investment for the PAnMBR will be $4,109,307.

Microalgae: Algae and Cyanobacteria

26.0 Closed Photo Bioreactor

The use of algae and cyanobacteria in closed photo-bioreactors generally required HRT around 10 days [71]. At the same time the energy for illumination for algae is up to 10 times higher than PPB with intensities around 100 – 200 W m^{-2} required [96]. Cyanobacteria can grow efficiently from 6 – 25 W m^{-2} [85]. Algae and cyanobacteria rely on UV-VIS light for the photosynthesis. The use of IR LEDs for PPB (> 800nm) saves up to 70% energy per photon compared to the UV-VIS range (200 – 700nm) [28]. The reactor internals for light supply will be comparable to the PAnMBR with PPBs. However the reactor volume for algae and cyanobacteria will be 10 times the size of the PPB reactor whereby and the illumination intensity for algae will be 10 times higher compared to PPB. The tank volume at 1730 m^3 d^{-1} will be 17300m^3 @ \$2000 m^{-3}. This option is not considered as economically feasible and detailed economic calculations are not provided.

27.0 Open Raceway Pond/High Rate Algae Pond as sole treatment

Open raceways are better suited for wastewater treatment purposes (Figure 17). However, the treatment of red meat processing wastewater stream in a raceway pond or HRAP is not practical without pre-treatment, such as removal of organics using a CAL.

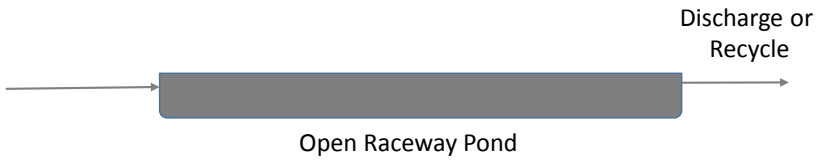

Figure 17: Process flowsheet representing a microalgae in a raceway pond.

The following capital and operational costs calculation is based on a COD loading rate of 130 g $m^{-2}d^{-1}$, HRT of 10 days [97] and biomass production of 24.5 g.$m^{-2}d^{-1}$ as described by [70]. The same source described average COD, TKN and TP removal efficiencies of 77%, 88% and 0%. We assume 50% of P removal at this point. The major removal of nitrogen is likely due to nitrification, driven by oxygen produced by the microalgae. A part of the formed nitrate is likely to be denitrified. Nitrate is likely to be present in the effluent. Based on these efficiencies the effluent load of COD, N and P will be 3979 kgCOD d^{-1}, 112 kgN d^{-1} and 57 kgP d^{-1}. Based on the COD load and a desired SRT of 10 days the flow to the centrifuges would be only 13 m^3 d^{-1} which results in very limited biomass recovery.

Another major drawback is that the influent needs to be diluted in order to achieve the design surface load of 130 gCOD $m^{-2}d^{-1}$. This would affect the HRT and requires larger pond surface area. A dilution of 2.3 is needed which results in 130,077 m^2. Additionally, the evaporation loss in tropical regions can be up to 10 L $m^{-2}d^{-1}$ [98] resulting in up to 1330 m^3 water loss per day. We assumed 2.5 L $m^{-2}d^{-1}$ over the year (332 m^3d^{-1}). This water has to be added on top of the dilution water. In the worst case scenario a dilution stream of up to 5309 m^3 d^{-1} would be required. The recycling of effluent is critical due to rather high COD, N and P residue.

However in case of effluent recycling the flow to the centrifuges would be around 4000 m^3d^{-1}. In any case fresh dilution water is required also with focus on increasing salinity over time. The bulk water price in Brisbane is \$2,547 ML^{-1}.

The biomass production of this treatment option is 1190 t $year^{-1}$ based on a productivity of 24.5 $g.m^{-2}d^{-1}$.

Centrifuges are generally used to concentrate biomass from raceway ponds. We apply 0.3kWh $kgDS^{-1}$ and polymer usage of 4.7kg tDS^{-1} at \$1.4 kg^{-1}.

Table 36: Summary of capital costs for the raceway pond.

	Basis	Estimated Capital
Raceway pond	133,077m^2 @ 0.3m depth	\$638,769
Centrifuge	500,000 @4.0 tDS d^{-1}	499,038
Installation and ancillaries		\$68,843
Engineering costs	10% of capital	\$120,665
Total estimated capital		\$1,327,000

Table 37: Summary of operating costs for the raceway pond.

	Basis	Estimated Expenditure
Operator support	0.35 FTE at \$80,000	\$28,000
Vessel and pipe maintenance	2-4% capital	\$24,133
Mixing, paddle wheels	6.4 kJ $m^{-3}d^{-1}$	\$2,623
Centrifuge and polymer dosing	0.3kWh $kgDS^{-1}$ and 4.7 kgPoly tDS^{-1}	\$45,714
Illumination energy demand	Sunlight	\$0
Water	Only evaporation loss	\$309,289
Revenues organic fertiliser	To be determined, assume \$336 t^{-1}*	-\$409,759

***biomass produced is around 1190 t $year^{-1}$. In order to be cost neutral 1 t has to sell for \$336.

Another option would be the focus on nutrient removal withy microalgae e.g. after a CAL (Figure 18. We assume that sufficient COD is present in the CAL effluent (4844 kgCOD d^{-1}) wastewater and external addition of CO_2 is not necessary.

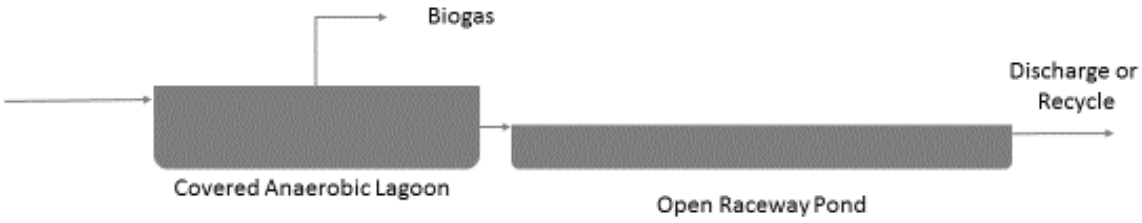

Figure 18: Process flowsheet representing a covered anaerobic lagoon followed by microalgae in a raceway pond.

This option would result in the following capital and operational costs. The summaries of capital and operational costs are shown in Table 38 and Table 39. This configuration would produce 354 t biomass per year. The potential revenues of this product were not included in the calculations. However, if we assume a value of \$400 t^{-1} this would add a revenue of \$141,643 $year^{-1}$.

Table 38: Summary of capital costs for a covered anaerobic lagoon followed by a raceway pond

	Basis	Estimated Capital
Covered anaerobic lagoon	6912 m^2 area and 6 m depth	\$907,000
Cogeneration unit	562 kW @ \$1,500 kW^{-1}	\$1,055,000
Raceway pond	57,667m^2 @ 0.3m depth	\$276,800
Installation and ancillaries		\$140,024
Centrifuge	500,000@4.0 tDS	\$77,000
Engineering costs	10% of capital	\$245,582
Total estimated capital		\$2,701,406

Table 39: Summary of operating costs for covered anaerobic lagoon followed by a raceway pond

	Basis	Estimated Expenditure
Operator support	0.35 FTE at \$80,000	\$28,000
Vessel and pipe maintenance	2-4% capital	\$54,028
CAL energy demand	0.01 kWh $m^{-3}d^{-1}$	\$19,000
Electricity generation from co-gen	\$0.1 kWh^{-1}	-\$616,000
Raceway pond	0.0018kWh$m^{-3}d^{-1}$	\$1,137
Centrifuge and polymer dosing	0.3kWh $kgDS^{-1}$ and 4.7kgPoly tDS^{-1}	\$17,015
Renewable energy credits	\$0.034 kWh^{-1}	-\$216,000
Water	Only evaporation loss	\$134,025
Total estimated operating		-578,795

*potential revenue from biomass is \$141,643 year-1 at \$400 t-1.