FINAL REPORT

Problem to Profit: Developing a sustainable feed base from agricultural wastes using single cell protein

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EXECUTIVE SUMMARY

Purple Phototrophic Bacteria (PPB) are an emerging technology that enables the treatment of wastewater streams while producing potentially valuable feed or feed additives. In this technology, the removal of organics, nitrogen and phosphorus from wastewater occurs anaerobically in the presence of infra-red (IR) irradiation. Nutrients and organics are assimilated and/or accumulated by the PPB, which convert soluble compounds into a harvestable biomass with high protein content.

Previous research (2016/1023) established the proof-of-concept by growing PPB on RMP wastewater in laboratory batch tests. Research presented in the current report focused on the development of reactor designs for a continuous laboratory process. The most critical initial decision in PPB technology development is the PPB growth mode. PPB can grow in suspension, similar to an algae raceway. This growth mode would have simple and lower cost reactor designs, but much higher product harvesting costs. Product quality may be lower if non-PPB solid particulates present in RMP wastewater are captured with the product. Alternatively PPB can grow as part of attached biofilms. Biofilm reactors are more complex and the harvesting mechanisms require further development; however biofilms are highly concentrated compared to cell suspensions and are generally higher quality as solid contaminants are not captured within the biofilm.

PPB production from RMP wastewater was successfully achieved using both attached growth (such as biofilms) and using suspended growth modes. Attached growth resulted in a relatively consistent PPB product with high protein content (approx. 65%). Suspended growth resulted in more variable PPB product quality between 30% and 70% crude protein, depending on the accumulation of wastewater particulates within the settled PPB product. While PPB product quality using attached growth is higher and more consistent when compared to suspended growth, the attached growth reactors are more complicated and manual biofilm harvesting may be labour intensive. At larger scale, the suspended growth process could be implemented as a constructed bioreactor (using similar design principles to the lab reactors) or as a lagoon/raceway type system where a light filtering cover is used to supply the IR spectrum of sunlight. In this regard, the multi-chamber process is flexible enough to be implemented as either a higher-cost lower-footprint bioreactor option or a low-cost high-footprint lagoon option. With either technology, capture of nitrogen in the PPB product and subsequent conversion to microbial protein was limited, largely attributed to the form of nitrogen entering the reactors. The research focus is now optimizing PPB yields, through the use of pre-fermentation and/or pre-conditioning of waste streams.

There are multiple options for implementing PPB technologies into the wastewater treatment process at RMP, such as application directly after primary treatment or application after secondary treatment using a CAL. In general, PPB technologies will reduce biogas revenue as a portion of COD is redirected from biogas production is consumed during PPB growth. However, biogas production is only reduced by 20% and this reduction also occurs in a conventional BNR process where COD is consumed during nitrogen removal. There is substantial potential to further optimize PPB technologies by reducing PPB production costs, this work is expected to target illumination costs through the use of filtered sunlight and/or harvesting costs through the use of biofilm or granular technologies.

For PPB to economically feasible the PPB biomass has to be marketed as a high value organic fertiliser and/or as protein-rich feed additive. Based on this report, PPB could have a similar or higher value 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. However, PPB technologies become most attractive when the PPB product is marketed as a fish meal substitute in aquaculture feeds. Current market prices for Fish meal exceed $2,000 AUD (May, 2018). The highest value assigned to PPB is $1,200 per dry tonne and corresponds to value recovery exceeding $3,500 per ML of wastewater treated.

Protein from Solid Waste

Paunch solid waste is a problematic solid waste stream at many Australian RMP. Paunch solid waste was considered as a feed stream for PPB production, however the high COD:N:P ratio of the paunch solid waste indicates that paunch does not contain sufficient nutrients for a high yield waste-to-protein technology such as PPB. Additionally, the paunch solid waste contained >95% particulate organic material, and this material is unlikely to be metabolized by PPB in the particulate form. However, the high solids content and high carbon content of paunch solid waste may be suitable for alternative waste-to-value technologies such as mushroom fermentation. Mushroom fermentation is an emerging technology that has been investigated for application to municipal solid waste. Potential advantages of mushroom fermentation include:

- Production of a relatively cheap source of high quality food protein using degradable cellulosic wastes
- Production of nutritionally enhanced dietary supplements/mushroom nutraceuticals (Mushroom Biotechnology),
- Bioconversion of difficult lignocellulosic materials into highly degradable bioenergy feedstocks
- Bioconversion/bioremediation of environmental contaminants (i.e. heavy metals).

Initial results show strong growth of oyster mushrooms on sterilized paunch, with moderate growth also observed by aspergillus and enoki. These results could not be replicated on raw paunch with little or no growth observed. The results demonstrate that fungi are able to utilize paunch as a substrate for growth, however the process may be inhibited by native bacteria within the paunch or there may be a requirement for a physical pre-treatment (i.e. steam explosion) to change the structure of the paunch fibres and increase the bioavailability of the material. The requirement for sterilization pre-treatment in the initial tests is a very significant practical challenge to be considered when assessing viability of the technology. This may be addressed through trialing alternative mushroom species; however a solution has not been confirmed at this time.

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ABBREVIATIONS

AAR	Anaerobic Ammonium Removal
AD	Anaerobic Digestion
AMPC	Australian Meat Processer Corporation
BNR	Biological Nutrient Removal
CAL	Covered Anaerobic Lagoon
COD	Chemical Oxygen Demand
CRC	Cooperative Research Centres
DAF	Dissolved Air Flotation (tank)
FOG	Fat, Oil, Grease
HRT	Hydraulic Residence Time
IR	Infra-red
MCPB	Multi Chamber Photo Bioreactors
N	Nitrogen
NH3	Ammonia
NH4-N	Ammonium nitrogen
NO2-N	Nitrite Nitrogen
NO3-N	Nitrate Nitrogen
P	Phosphorus
PAnMBR	Phot Anaerobic Membrane Bioreactor
PHA	Polyhydroxyalkanoates
PO4-P	Phosphate Phosphorus
PPB	Purple Phototrophic Bacteria
QUU	Queensland Urban Utilities
RMP	Red Meat Processor
SCP	Single Cell Protein
TAN	Total Ammonical Nitrogen
TKN	Total Kjehldahl Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids

1 INTRODUCTION

1.1 Summary

Waste-to-value technologies are a novel concept where waste treatment technologies are replaced with value-add alternatives that allow capture and recovery of components such as carbon, nitrogen and phosphorous. Purple phototrophic bacteria (PPB) are a form of value-add technology where carbon, nitrogen and phosphorus are captured through microbial growth and synthesized into microbial protein, resulting in an organic solid concentrate stream suitable for recovery and export. PPB have been applied to domestic wastewater treatment, but have not previously been applied to meat processing wastewater. This project will apply purple phototrophic bacteria in multiple scales, from small-scale proof of concept, through to continuous laboratory-scale operation, to study the treatability of red meat processing wastewater with focus on effect of solid COD as well as fat content. Another major component is a full value proposition for complete resource recovery from red meat processing wastewater streams including the full utilisation of the produced PPB biomass as organic fertiliser and/or as a potential feed additive. This offers the ability to value-add nutrients, and make wastewater treatment revenue generating beyond the value propositions demonstrated through current processes. This option offers the potential of waste-free wastewater treatment with an additional revenue stream rather than sludge disposal costs.

The project is a continuation of AMPC project 2016/1023 and will build on recent industry research and development in this area, including:

- 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
- A.ENV.0164 Feasibility study into the application of anaerobic ammonium removal technology for wastewater treatment at red meat processing facilities
- 2013/5024: Robust membrane systems for enhanced primary treatment and energy recovery of abattoir waste water

1.1 Background

Previous AMPC funded research has demonstrated that slaughterhouse wastewater has a relatively high ratio of organics to nitrogen (A.ENV.0131, A.ENV.0151), and a very high biodegradability (A.ENV.0133, 2014/4007), this has led to the application of traditional waste treatment technologies such as covered anaerobic lagoons and conventional biological nutrient removal processes. While effective, these conventional technologies:

i) Can occupy very large footprints and are not suitable for all red meat processers
ii) Have known operational problems, such as scum formation – leading to variable performance and in some cases equipment damage.

iii) May require complex hazardous zoning equipment and biogas safety management plans
iv) Have potentially high operating costs, due to aeration energy and waste sludge disposal

RMP wastewater is potentially suited for an entirely novel waste-to-value process utilising infrared light to select for purple phototrophic bacteria (PPB). Under these conditions the PPB utilise organics, nitrogen, and phosphorous for microbial growth instead of metabolising these compounds for energy. In this process, energy for growth comes from light, instead of conversion of organics or nitrogen. This results in very high biomass yields, with essentially all the organics (carbon) and nitrogen partitioned into microbial biomass instead of being released as gas (carbon-dioxide, methane, nitrogen etc). PPB biomass typically contains in the order of 65% protein content, 25% carbohydrates, remainder ash and lipids (on a dry weigh basis). When compared to existing technologies:

- The PPB process does not produce a gasoues product (such as biogas), this is a major difference compared to carbon removal processes such as covered anaerobic lagoons and reduces the complexity of process design, as there are no hazardous zone requirements.
- The PPB process does not require energy intensive aeration and this is a significant improvement over conventional nitrogen removal processes, such as activated sludge processes.
- The PPB product can be harvested as a valuable product and this is another key difference between PPB processes and conventional activated sludge processes, where activated sludge is a waste product requiring disposal.

A major advantage of PPB is the ability to produce a high-protein microbial product. The microbial protein has the potential to be used as a feed material in livestock or aquaculture industries, however the ability to generate value from the microbial product is subject to both product testing/market development and potential regulatory hurdles. Regulatory issues are a potential hurdle, but are not seen as a major disruptor or barrier. Current irrigation practices in the RMP industry where treated wastewater is irrigated to grow rye grass, which is harvested and used as cattle feed demonstrate that waste-to-feed pathways exist within the sector.

1.2 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} COD, with high nitrogen and phosphorous levels also present. While potentially expensive, the removal of these contaminants is necessary in order to comply with water discharge regulations. Wastewater contaminant 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.

Biogas processes such as covered anaerobic lagoons (CAL) and high-rate anaerobic membrane processes (AnMBR) can generate revenue through onsite energy production (payback 2-5 years), however biogas processes leave residual nitrogen (200-400 mgN L^{-1}) and phosphorous (up to 50 mgP L^{-1}) in the wastewater. The wastewater can be irrigated and used to core ryegrass or maize, but this generally requires very large land footprints (100-200 ha) and is not suitable for all RMP. Alternatively, wastewater can be 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); again, not all RMP all have to sewer networks. 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 Anammox allow economic removal of N, and are nearer to market, but do not offer the possibility for nitrogen or alternative 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 convert COD, N and P into a single value-add product.

1.2 Summary of Waste Production at Red Meat Processing facilities

Australian red meat processing facilities have potential to generate large volumes of wastewater and solid waste rich in organic contaminants and nutrients (Johns 1995, Liu and Haynes 2011). While potentially expensive, the removal of these contaminants is necessary in order to comply with water discharge regulations. Therefore red meat processing facilities are strong candidates for advanced treatment processes aimed at removal and/or subsequent recovery of energy, nutrient, and water resources.

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. A comparison of Table 1 and Table 2 shows that wastewater from Australian slaughterhouses is concentrated by international standards, both in regards to organic contaminants (COD) and nutrient (N and P). It is also important to note that the COD:N:P ratio of Australian RMP is 100:2.5:0.5, this is also high by international standards (with the exception of US) and indicates there may be excessive carbon and insufficient nutrients for complete conversion to microbial protein.

Table 1 Composition of combined wastewater at Australian slaughterhouses compared with literature values

	Volume m^{3} day^{-1}	TCOD mg L^{-1}	sCOD mg L^{-1}	TS^{b} mg L^{-1}	FOG mg L^{-1}	N mg L^{-1})	P mg L^{-1}
Literature Concentration^{a}	-	2,000-10,000	-	500-2,000	100-600	100-600	10-100
Site A	2420	12,893	1,724	8,396	2,332	245	53
Site B	3150	9,587	1,970	4,300	783	232	50
Site C	2110	10,800	890	7,530	3,350	260	30
Site D	2150	12,460	2,220	7,400	1,200	438	56
Site E	1600	10,925	1,195	6,118	1,569	272	47
Site F	167	7,170	1,257	3,806	1,915	182	27

a. Based on (Cowan et al. 1992, Johns 1995, Mittal 2004, Tritt and Schuchardt 1992)
b. Literature values are TSS (mg/L), study values are TS (mg/L)

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 mg L^{-1}	NH_4-N mg L^{-1}	TP mg L^{-1}
Borja et al. (Borja et al. 1994)	Spain	5,100	-	-	310	95	30
Caixeta et al. (Caixeta et al. 2002)	Brazil	2,000-6,200	-	40-600	-	20-30	15-40
Li et al. (Li et al. 1986)	China	628-1,437	-	97-452	44-126	25-105	10-16
Manjunath et al. (Manjunath et al. 2000)	India	1,100-7,250	-	125-400	90-150	-	8-15
Martinez et al. (Martinez et al. 1995)	Spain	6,700	2,400	1,200	268	-	17
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	30	15
Sachon (Sachon 1986)	France	5,133	-	897	248	-	22
Sayed et al. (Sayed et al. 1987)	Holland	1,500-2,200	-	-	120-180	-	12-20
Sayed et al. (Sayed and De Zeeuw 1988)	Holland	1,925-11,118	780-10,090	-	110-240	-	13-22
Stebor et al. (Stebor et al. 1990)	US	4,200-8,500	1,400	100-200	114-148	65-87	20-30
Thayalakumaran et al. (Thayalakumaran et al. 2003)	NZ	490-2,050	400-1,010	250-990	105-170	26-116	25-47

1.3 Wastewater Treatment

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). 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.

A general structure of wastewater handling practices is presented in Figure 1 and includes screening to reduce total suspended solids, dissolved air flotation (DAF) as a pre-treatment to remove fat, oil and grease (FOG) and further reduce 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 in tropical and equatorial temperate zones and engineered reactor systems (including activated sludge and UASB reactors) are commonly used in polar equatorial temperate zones. Anaerobic lagoons 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.

In the anaerobic step, proteins will be converted to biogas and the organic bound nitrogen will be realized 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, existing technologies can require energy intensive aeration steps and carbon chemical addition. Anaerobic ammonium removal technology is an emerging option to replace traditional nitrogen removal technologies at lower cost, however the focus of AAR is still removal. Single Cell Protein technologies, such as algae or PPB are alternative technologies designed to capture and transform nitrogen into a valuable product.

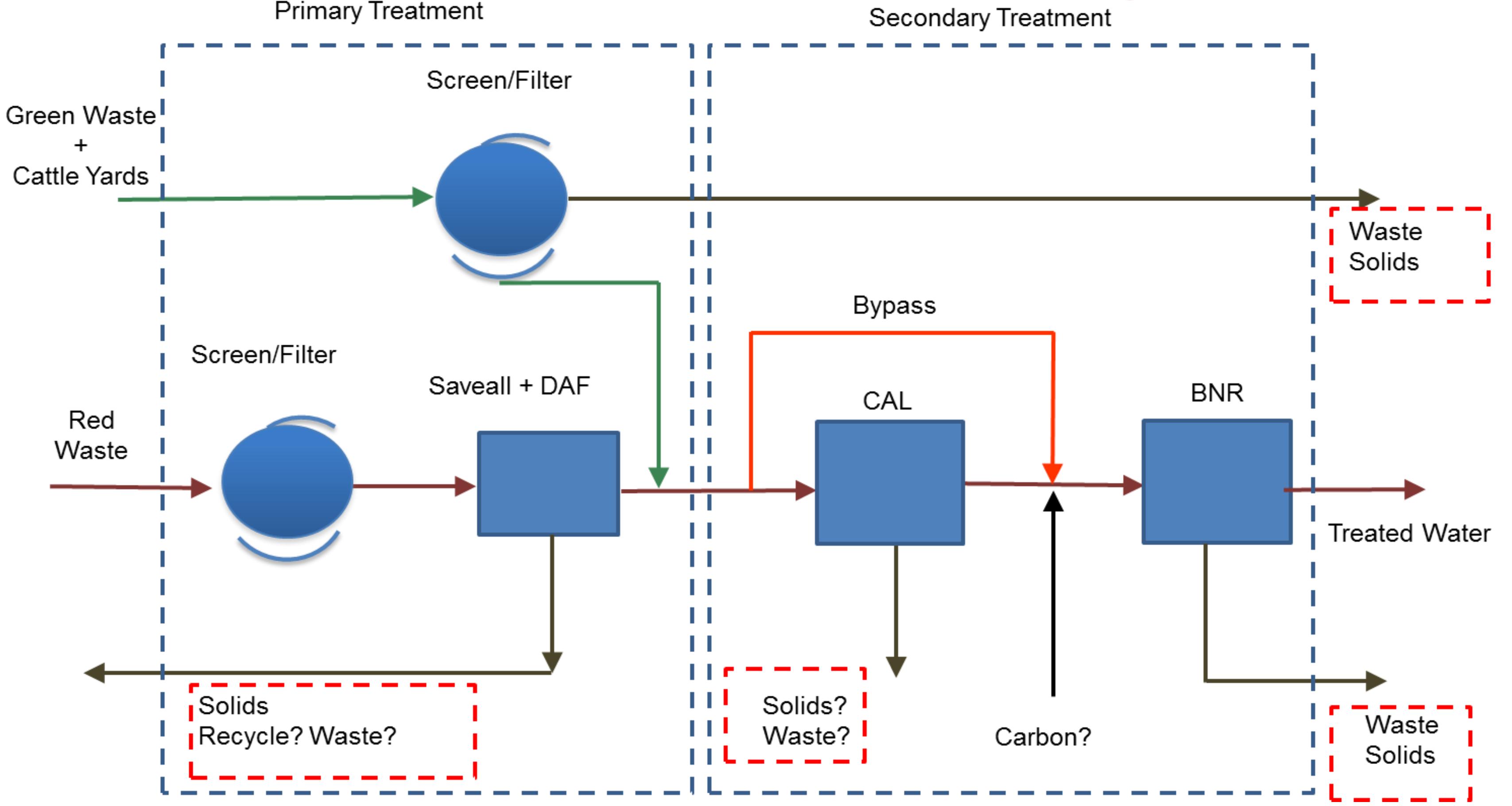


Figure 1: Example of waste handling process at Australian red meat processing facilities and major sources of organic solid waste (not including dead animals or packing wastes).

1.4 Solid Waste Collection and Handling

Solid waste originates from processing areas including paunch, manure, screenings (not rendered), DAF sludge, aerobic wastewater sludge, contaminated cardboard and condemned/dead animals. Cattle paunch in particular is a major waste produced at cattle slaughterhouses and is comprised of partially digested cattle feed, mainly containing grass and grain. The volume and composition of paunch waste varies according to individual animals and site handling practices but is reported at approximately 60 kg of wet paunch waste per animal (5-7 kg solids), corresponding to approximately 10% of the total weight of the live animal.

Current disposal methods for paunch and other solid wastes are largely based on composting, land disposal or landfilling. Direct land disposal is generally facing increasing regulation with application permits often required. Onsite composting is generally effective, but can require a large footprint and is a high-risk activity in terms of odour generation. Landfill can be a high-risk disposal method due to the landfill space availability and rapidly increasing landfill gate fees. Therefore, alternate disposal methods are required – with a preference for disposal methods that facilitate value recovery in the form of energy, nutrient fertilizers/organic mulches or other value add products.

AMPC has previously funded a series of research projects investigating the viability of using paunch waste as a boiler fuel (A.ENV.0110, A.ENV.120-123). Outcomes from these projects demonstrated some success when adding paunch to co-fuel the trial boiler, when the paunch was dewatered to TS content above 30% and mixed with a sawdust fuel at TS of 60%. The volumes of paunch combusted in these trials were much lower than the ratio of paunch produced at a typical meat processing plant, and the impact of paunch TS and paunch type (grass, grain etc.) were not reported. While there were some impacts on boiler efficiency, these short-term trials (approx. 3 hours) do demonstrate significant potential for paunch treatment through combustion-based technologies. However, there are a limited number of slaughterhouse's with the infrastructure to adopt the multi-fuel boiler approach.

Anaerobic digestion (AD) is an alternative approach to recover energy from paunch that was investigated through several AMPC/MLA projects (ENV.0068, A.ENV.0099, A.ENV.0155). The AD projects were successful at reducing the mass of paunch waste (60%) and recovering methane rich biogas (7 GJ/dry Tonne), however many conventional forms of AD were not considered economically attractive when applied to paunch. This was largely due to slow treatment times and high cost infrastructure requiring large capital investments. In both approaches, the economic success of the process appeared to be driven by a reduction in disposal costs, rather than the value of energy recovered.

Solid waste handling processes at RMP are generally include screening and dewatering technologies to reduce the volumes requiring disposal offsite. A brief summary of dewatering units and operational considerations are shown in Table 3. In plants using wet dump paunch handling processes, the solids content of paunch waste typically varies in the range of 5,000 mg/L TSS to 30,000 mg/L TSS prior to solids capture. The reported effectiveness of solids and nutrient capture during paunch dewatering processes varied between studies (MLA/AMPC 2012, 2013, MLA/AMPC. 2013). Generally 60-80% of paunch solids will be captured in the dewatered cake, however this may be increased to over 95% by adding chemical agents.

Recovery of phosphorus and potassium during dewatering is generally poor with 75-90% of P and K remaining in the filtrate after solids removal and transported to the wastewater stream. Recovery of nitrogen is more variable with 50-90% of N remaining in the filtrate wastewater. Therefore, while paunch dewatering units as an effective strategy for reducing solids they are not an effective strategy for reducing nutrient loads. Capture of both nitrogen and phosphorous can be significantly improved by adding chemical agents during dewatering, however the chemical costs can be substantial, and currently the captured material is a waste product requiring disposal.

Table 3: Summary of common equipment used for solids recovery from paunch

	Static Screen	**Rotary Screen**	**Screw Press**	**Degritting Hydrocyclone**
Capital Cost	Low ($15-20k)	Low ($15-20k)	Moderate ($50-80k)	Moderate ($50-80k)
Operating Cost	Low	Low	Moderate	Low
Life expectancy	Long	Long	Component replacement(s) after 10 years. Screens are subject to wear and may require replacement after 2-3 years	Moderate life
Application Area	Gross and Paunch Solids	All Solids	Paunch and Manure Solids	Stockyard Grit
Solids Cake	Wet	Wet	Dry (up to 50% solids)	Wet
Operating Weakness	Susceptible to hydraulic overloading and blockage	Susceptible to hydraulic overloading	Susceptible to damage from boluses or a lack of fibrous solids; damage from metallic objects in waste streams	Susceptible to blockage from paunch balls

1.5 Purple Phototrophic Bacteria

Purple phototrophic bacteria (PPB) are naturally occurring microorganisms 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 contain photosynthetic pigments that allow them to generate energy from light rather than from other chemicals (Basak and Das 2007), specifically, PPB utilize IR light. In the waste-to-value area, PPB represent a form of single cell protein technology (SCP). Single cell proteins are 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 (Matassa et al. 2015). The composition of several common species of PPB is shown in Table 4 with average microbial protein contents of 60-65% on a dry weight basis.

The very high protein content makes PPB a key candidate for organic fertiliser applications Xu (2001) and animal feed applications, particularly fish (Kobayashi and Tchan 1973) and poultry Ponsano et al. (2004). In addition to high protein, PPB biomass contains a variety of useful products such as, vitamins, carotenoids, and ubiquinone (Takeno et al. 1999). While these compounds are potentially very high value, additional extraction and purification steps are required.

In wastewater applications, PPB will remove organics, nitrogen and phosphorus from the wastewater simultaneously in ratios of approximately 100:8:1.3. In this regard, PPB have the potential to be applied as a stand-alone treatment process, dependant on wastewater composition. PPB have been applied successfully for growth on 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). However, development of the technology on other wastewater streams is relatively limited.

Table 4: General composition of several PPB species.

	R. capsulatus[1]		Rps. Gelatinosa[2]		R. gelatinosus[3]	
	% 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
Crude fat	9.9	3.7	n.d	3.7	0.5	0.2
Soluble carbohydrates	20.8	3.5	n.d	3.5	25.6	4.3
Crude fiber	2.9	-	n.d	-	n.d	-
Ash	5.3	-	n.d	-	4	-
Total	-	17.4	-	18.1	-	15

adapted from [1] (Blankenship et al. 1995a), [2] (Shipman et al. 1975), [3] (Ponsano et al. 2004), [4] (Adedokun and Adeola 2005), n.d = not determined.

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 (Kim et al. 2004). 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 (Azad et al. 2004). 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 100:2.5:0.4 – 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.

2 PROJECT OBJECTIVES

This project is a continuation of 2016/1023 "Purple phototrophic bacteria for resource recovery from red meat processing wastewater". The project is to be delivered in 2 subprojects:

2.1 Sub-project 1: Continued Development of PPB for Red Meat Processing Wastewater.

The remaining research objectives to be developed in the current project (2016-1023) are:-

1. Can PPB be selectively enriched from slaughterhouse wastewater using only infrared light as driver? What effluent nitrogen, phosphorous, and organics levels can be achieved?
2. What is the resulting microbial material, and digestability of the product?

This represents the end-point for the existing work in year 1 (2015/2016 FY).

This project develops the current research into the following extension project over the following two years:

3. Can a continuous photo-bioreactor process be developed based on PPB?
4. What is the potential to design a process that enables production of high-purity microbial protein for alternative uses?
5. What is the market justification and scope for products

This represents the end-point for the project and is expected to lead to pilot application in follow up projects.

2.2 Sub-project 2: Expansion of SCP technologies for solid waste treatment.

Sub-project is a preliminary proof-of-concept assessment on the application of waste-to-protein technologies for RMP solid wastes, specifically paunch solid waste. Objectives include:

1. To screen emerging single cell protein (SCP) technologies, including the AMPC PPB platform for suitability to meat processing solid wastes.

2. Assess basic digestability of solid waste for SCP, determine the composition of the SCP product, and product applications.
3. Evaluate pre-treatment requirements for application of SCP to red meat processing solid wastes.
4. Develop a cost-benefit analysis of SCP compared to other solid waste management strategies appropriate for red meat industry applications.

This represents the end-point for the project and is expected to lead to further development and pilot application in follow up projects.

3 METHODOLOGY

3.1 Analytical Methods

3.1.1 Standard physical-chemical analysis

Table 5 provides a summary of analytical methods used in this project. For measurement of soluble COD (sCOD), TAN and PO_4-P, samples were centrifuged (5 min at 2,500 x g) and filtered through a syringe filter (0.45 µm PES membrane) prior to analysis. For total COD (tCOD) and total nutrients and metals, samples were analysed as collect with no pretreatment.

Table 5: Summary of general analytical methods used during the PPB project

Analysis	Description
Chemical Oxygen demand (COD)	Estimates the organic content of a sample. Also an order of magnitude estimate of chemical energy present in the sample (i.e. the energy released by each gCOD converted to CO_2 and H_2O by being chemically oxidised). Chemical oxygen demand (COD) was measured using Merck Spectroquant® cell determinations and a SQ 118 Photometer (Merck, Germany).
Total Solids (TS) Volatile solids (VS)	Total solids (TS) and volatile solids (VS) were measured in accordance with standard methods procedure 2540G (Franson et al. 2005).
Protein	Protein content was calculated according to Eding et al. (2006) (Eq.1). $g\ crude\ protein = gN\ x\ 6.25;$ $g\ protein \text{ expressed as } COD) = \text{crude } protein\ x\ 1.25; \qquad (1)$ Where; gN = TKN (mg L^{-1}) – NH4-N (mg L^{-1}).
Volatile Fatty Acids (VFA)	Individual VFAs (acetate, propionate, butyrate, valerate, and caproate) and alcohols (methanol, ethanol, and butanol, where relevant) were analysed with Agilent 7890A gas chromatograph with Agilent DBFFAP column.
Chemical Oxygen demand (COD)	Estimates the organic content of a sample. Also an order of magnitude estimate of chemical energy present in the sample (i.e. the energy released by each gCOD

Analysis	Description
	converted to CO_2 and H_2O by being chemically oxidised). Chemical oxygen demand (COD) was measured using Merck Spectroquant® cell determinations and a SQ 118 Photometer (Merck, Germany).
Total Kjeldahl Nitrogen and Phosphorous (TKN and TKP) Key Soluble Nutrients (NO_3^-, NO_2^-, amoniacal nitrogen, PO_4^-)	Nutrients (solid form and soluble). Nutrient content is related to resource recovery opportunity. Nutrient content may also impact downstream processing requirements (i.e. secondary treatment after AD). Total Kjeldahl nitrogen (TKN), total phosphorus (TP), total ammoniacal nitrogen (TAN), and phosphate-phosphorus (PO_4-P) were measured using a Lachat Quik-Chem 8000 Flow Injection Analyser (Lachat Instrument, Milwaukee).
Metals (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, & Zn)	Trace metals in a sample impacts on both the digestate quality and reuse potential. Trace metals may also provide resource recovery opportunities. Trace metals were measured using Inductively Coupled Plasma Optical Emission Spectrometry.
Alkalinity	Measured by titrating a volume of sample with HCl to end points of pH 5.7 and pH 4.3. Partial alkalinity was determined using the pH 5.7 endpoint and represents alkalinity contributed by hydroxides, ammonia, carbonate and bicarbonate. Intermediate alkalinity was determined as the difference between alkalinity to pH 5.7 and alkalinity to pH 4.3 and represents the contribution by organic acids. The alkalinity ratio (α) is defined as the ratio of partial alkalinity to intermediate alkalinity; with ratios <0.3 representing a healthy process (Ripley et al. 1986).
Dissolved Oxygen	Dissolved Oxygen was measured with an EASYSENSE O2 21 DO probe (Mettler Toledo, Australia). pH was measured using a AmpHel® pH Electrode (Hanna Instruments, Australia). Illuminance (Wm^{-2}) was measured with a UV-VIS & NIR light sensor (Stellarnet Blue Wave Spectroradiometer, Warsash Scientific, Australia).
pH	pH measurements used a calibrated Hanna pH sensor (HI2910B/5) and meter/transmitter HI 8614LN.
Gas composition	H_2, CH_4, CO_2, N_2, analysed using gas displacement, and gas chromatography with a Shimadzu GC-2014 equipped with a thermal conductivity detector (GC-TCD), electronic gas sampling valve (1 mL loop) and a HAYESEP Q 80/100 packed column (2.4 m length; 1/800 outside diameter, 2 mm inner diameter). The chromatograph injector, oven and detector temperatures are set at 75, 45 and 100 °C, respectively and Argon (99.99%) was the carrier gas at 28 mL min^{-1} and 135.7 kPa.

3.1.2 Total Pigments (carotenoids and bacterioclorophyll)

Carotenoids and chlorophyll are high value compounds that accumulate in PPB biomass and may be extracted as an enhanced value recovery opportunity. Establishing analytical methods for carotenoids and chlorophyll was a key task in the project. Method development included a detailed literature review and laboratory testing to implement the most suitable extraction and quantification methods compatible with the pigments encountered in PPB.

Analysis of total pigments (carotenoids and bacterioclorophyll) uses a colorimetric method. The method consists of pigments extraction with acetone and methanol (7:2), followed by measurement with a spectrophotometer. For extraction, samples for pigment analysis are initially frozen at -80°C. Samples are then thawed to 4°C and centrifuged at 1,800 g for 15 min (and 4°C) and the pigments from the pellet are extracted with acetone and methanol 7:2 while sonicating in ice for 10 minutes (modified from Van der Rest 1974 and Bóna-Lovász et al 2013 (Bóna-Lovász et al. 2013, Van der Rest and Gingras 1974)). The process is repeated until a colorless solution is obtained. The samples are centrifuged again and the pellet is removed. Total carotenoids in the supernatant are measured using a spectrophotometer at 475 nm to record absorbance (scale 0 to 1) using a Quartz cuvette. Bacteriochlorophylls in the supernatant are measured using a spectrophotometer at 771 nm to record absorbance (scale 0 to 1) using a Quartz cuvette. Pigment concentrations are calculated via the Beer-Lambert Law, assuming the Spirilloxanthin absorption coefficient (ε = 94000 $M^{-1}cm^{-1}$) for total carotenoids and using the Bacteriochlorophyll A absorption coefficient of 65300 $M^{-1}cm^{-1}$ respectivelly(Van der Rest and Gingras 1974).

3.1.3 Amino Acids

Analysis of amino acids is based on pre-hydrolysis of the samples to release amino acids into the liquid phase (i.e. not intra cellular matter) followed by HPLC.

3.2 Microbial Analysis

Biomass samples were stored for DNA extraction and microbial community analysis based on 16s amplicon sequencing. Analysis will be completed by the 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).

3.3 Productivity Assessments

Productivity represents new biomass formed by growing and multiplying cells and is expressed as mass per day per unit area of the illuminated surface (areal productivity) or per unit volume of the cell suspension (volumetric productivity). Productivity measurements in this project are primarily based on Areal productivity. Areal productivity is calculated using 2 methods. In Method 1 areal productivity is calculated by direct measurement of the harvested biomass. In Method 2, areal productivity is calculated using COD balances. In the absence of gaseous CO_2 and CH_4, it is assumed that all COD entering the system can only either be discharged via the effluent, or accumulate as settled and/or attached PPB biomass. Therefore, the difference in the values between influent and effluent COD (i.e. COD consumed) can be used as a basis for PPB productivity in the system – which may (or may not) be harvested.

3.4 Development of a Continuous Process

3.4.1 Reactor Design Overview

Initial proof-of-concept was demonstrated for the application of purple phototrophic bacteria (PPB) during treatment of RMP wastewater during project 2016/1023. Proof-of-concept was based on lab-scale batch testing. The current project aims to develop PPB as a continuous process and to maximising single cell protein production. After initial feasibility assessments, 3 reactor configurations were identified for further development:

a) Continuous Photo-membrane Bioreactor

b) Attached growth reactor with internal illumination via hollow tubes; and

c) Mixed chamber set-up with illumination from the outside.

The reactors were constructed and operation commenced in 2017. In addition to developing a continuous process for wastewater treatment, the project is exploring proof-of-concept to extend the SCP technology to red meat processing solid wastes.

3.4.2 Continuous Photo-membrane Bioreactor

Continuous photo anaerobic membrane bioreactors (PAnMBR) were previously developed and tested for PPB treatment of municipal (Hülsen et al. 2016) and poultry processing wastewater (ref). The PAnMBR uses a membrane to separate biomass and other particulates from the treated effluent. The previously developed PAnMBR is shown in Figure 2 and described in the following paragraph (values in parentheses refer to elements in the figure). The PAnMBR consisted of a 2 L rectangular acrylic tank equipped with a submerged flat sheet membrane (1) with 0.45µm pore size and 0.12 m^2 surface area (Kubota, Osaka, Japan). The reactor can be continuously fed wastewater using a Watson Marlow 120U/DM2 pump (6) (Wilmington, MA, USA). Level is controlled using a pressure sensor as level switch (GE 5000 Series Pressure Transmitter, Fairfield, CO, USA) at the site of the reactor (7) to control the effluent pump (WELCO peristaltic pump WPM1-S2AA-BP (8), Tokyo, Japan). Effluent removal was therefore semi-continuous. The PAnMBR is anaerobically illuminated at 20 W m^{-2} with illuminated surface to volume ratio of 20m^2 m^{-3} (IR 96 LED Illuminator for Night Vision Camera (2), St. Louis, MO, USA)). The reactor can be continuously mixed with an internal gas recycle of 6 L min^{-1} by a vacuum pump (specified above, (3)), Trenton, NJ, USA) via a condensate trap (4) through an air stone at the bottom of the reactor (5). The illuminance and wavelength profile of the reactor is measured at the outside with a UV-VIS & NIR light sensor (stellarnet blue wave spectroradiometer, Warsash Scientific, Australia). The pH and temperature are measured and recorded externally (TPS minichem pH (11), Brendale, QLD, Australia) (TPS minichem temperature (12), Brendale, QLD, Australia).

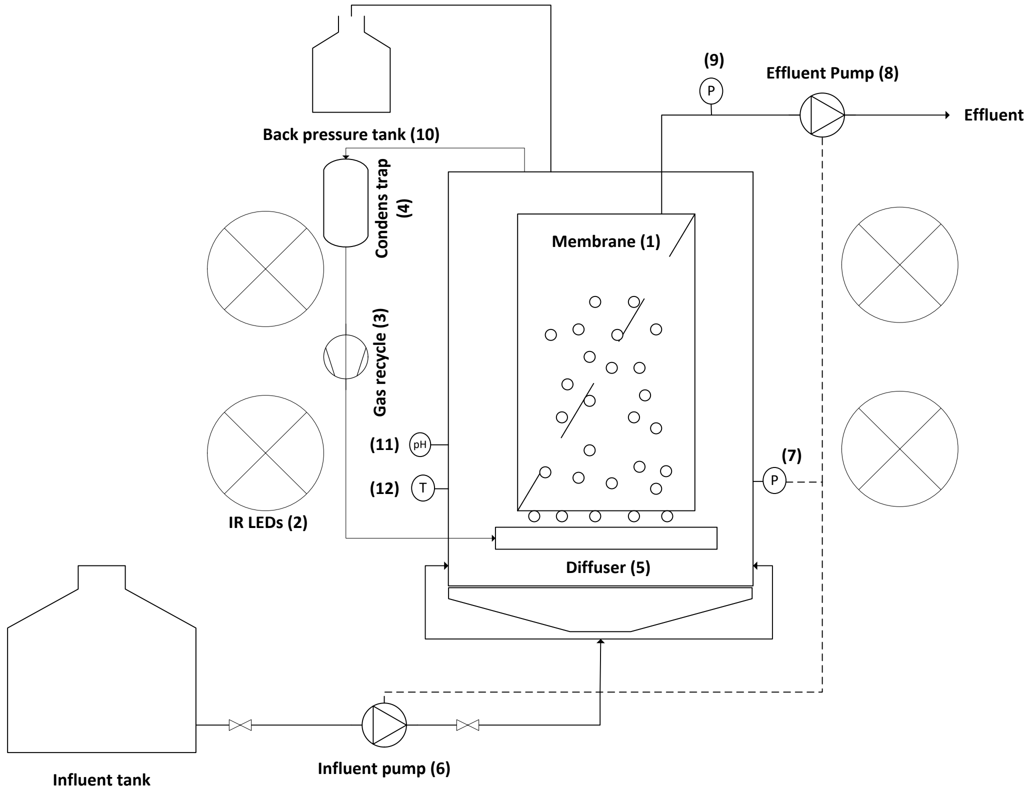


Figure 2: Schematic configuration of photo anaerobic membrane bioreactor

The PAnMBR was previously applied for PPB treatment of municipal (Hülsen et al. 2016) and poultry processing wastewater. These experiments operated for 250 days and showed simultaneous removal of COD, TN and TP. Analysis of the microbial product showed that PPB contributed >60% of the microbial biomass. While the PAnMBR successfully demonstrated the PPB process, the sludge harvesting was challenging and the product quality was variable. A PAnMBR treating poultry wastewater at lab-scale is shown in Figure 3, the purple color in the reactor shows that the PPB biomass is predominately suspended in the bulk liquid. PPB cells are very small (1.0 µm (Machulin et al. 2012)) which makes biomass harvesting and thickening more challenging. Biomass harvesting could be improved through flocculation followed by centrifugation, but would result in significant operational costs. Additionally, the PAnMBR captures all solids, including particulate contaminants in the raw wastewater. These particulates would also be captured with the PPB biomass during harvesting, this has the effect of reducing protein content and reducing product quality. Furthermore, chemical-physical up-concentration results in all solids being part of the sludge cake which in the case of PPB would reduce the overall protein content as the sludge cake would also include the inert fraction of the wastewater.

Another important aspect is that high volumetric loading rates (~6 gCOD L^{-1} d^{-1}) result in high biomass content in the reactor (>6g L^{-1}) which leads to shading and reduced light transfer efficiency. Particularly in the PAnMBR with suspended growth, this will affect the overall performance. In order to overcome the above-mentioned limitations with PAnMBR, alternative reactor configurations were developed.

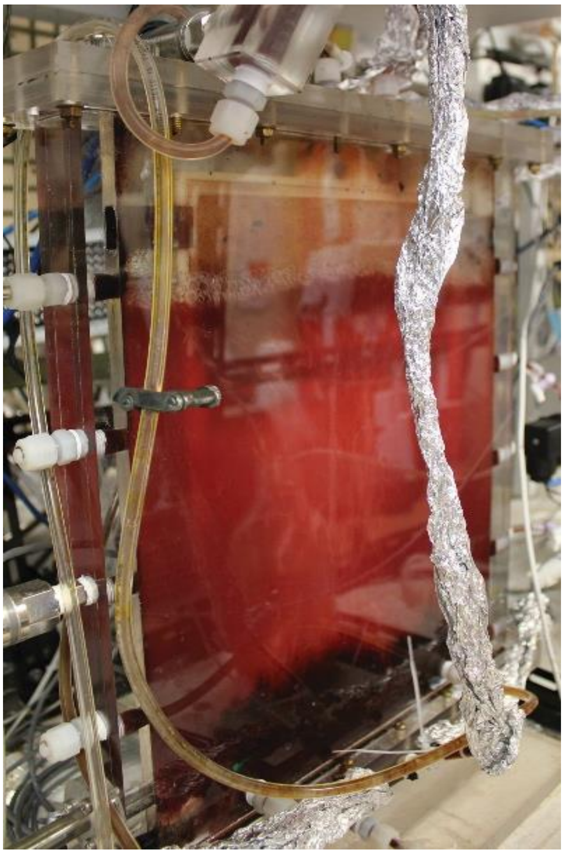


Figure 3: Photo anaerobic membrane bioreactor treating animal processing wastewater at lab-scale.

3.4.3 Continuous Attached Growth Photo Bioreactor

Attached growth bioreactors (developed previously) were adapted to overcome limitations with PAnMBR. The attached growth bioreactors contain hollow tubes constructed of clear acrylic. During operation, infra-red light is supplied from inside the tubes and this promotes development of a PPB biofilm on the outer-surface of the tubes. The biofilm limits the amount of infra-red light transmitted to the bulk liquid, this has the effect of promoting PPB growth on the tube surface and limiting PPB growth in the bulk liquid. PPB biomass is harvesting by periodically scraping the biofilm layer from the tube surface. This enables PPB to be harvested separately to any inert solids from within the reactor and produces a more consistent and higher quality product.

A 3L lab-scale attached growth reactor developed for this project is shown in Figure 4; a more detailed schematic of this reactor is shown in Figure 6. The 3 L reactor has hollow tubes inside the lid, which are illuminated from inside. A wiping system is installed to allow periodic biomass harvesting, the wiper is designed to scrap biofilms from the tubes which are withdrawn from the conical bottom of the reactor. A larger 1000 L pilot-scale attached growth reactor is shown in Figure 5. The pilot reactor was previously developed in projects funded by the CRC for Water Sensitive Cities.

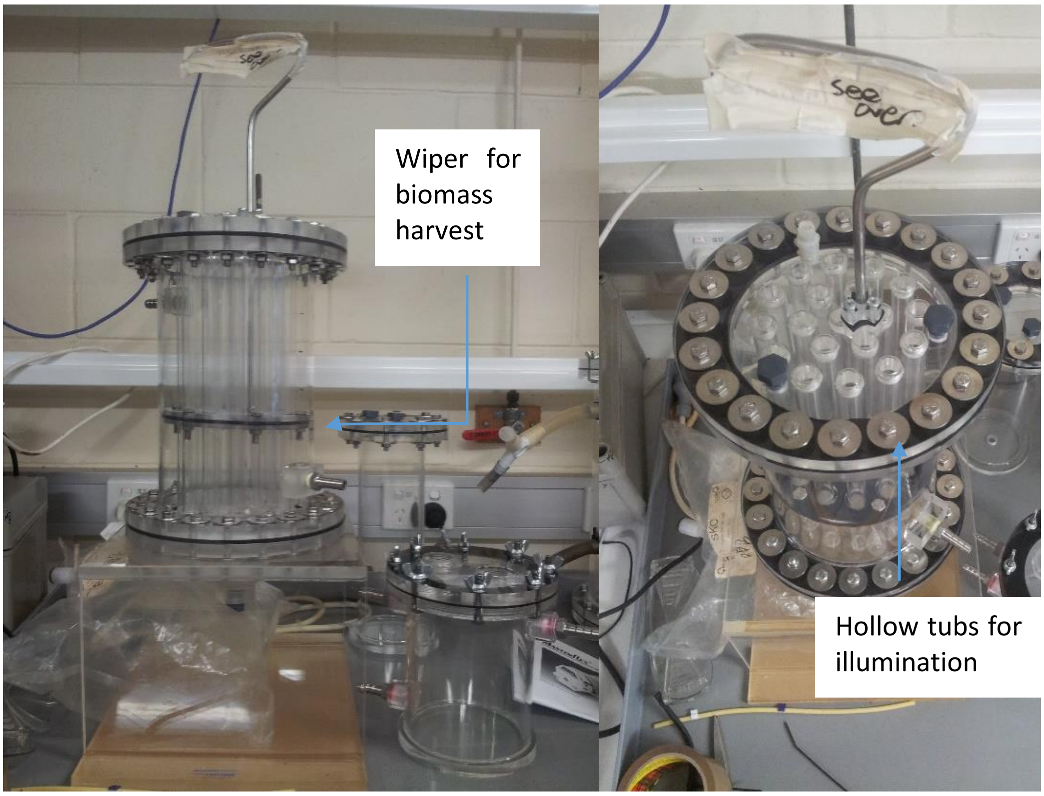


Figure 4: Attached growth photo bioreactor.

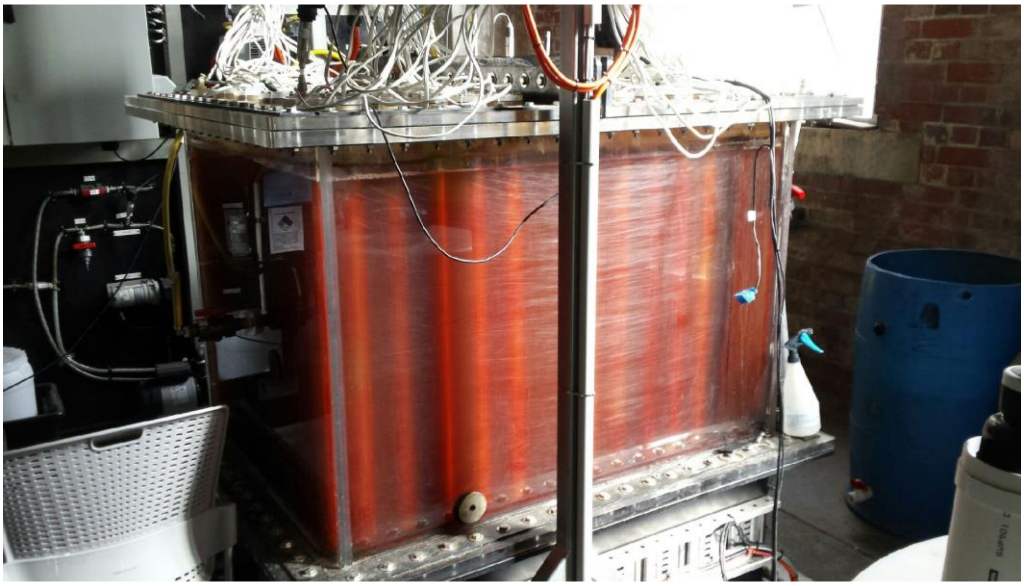
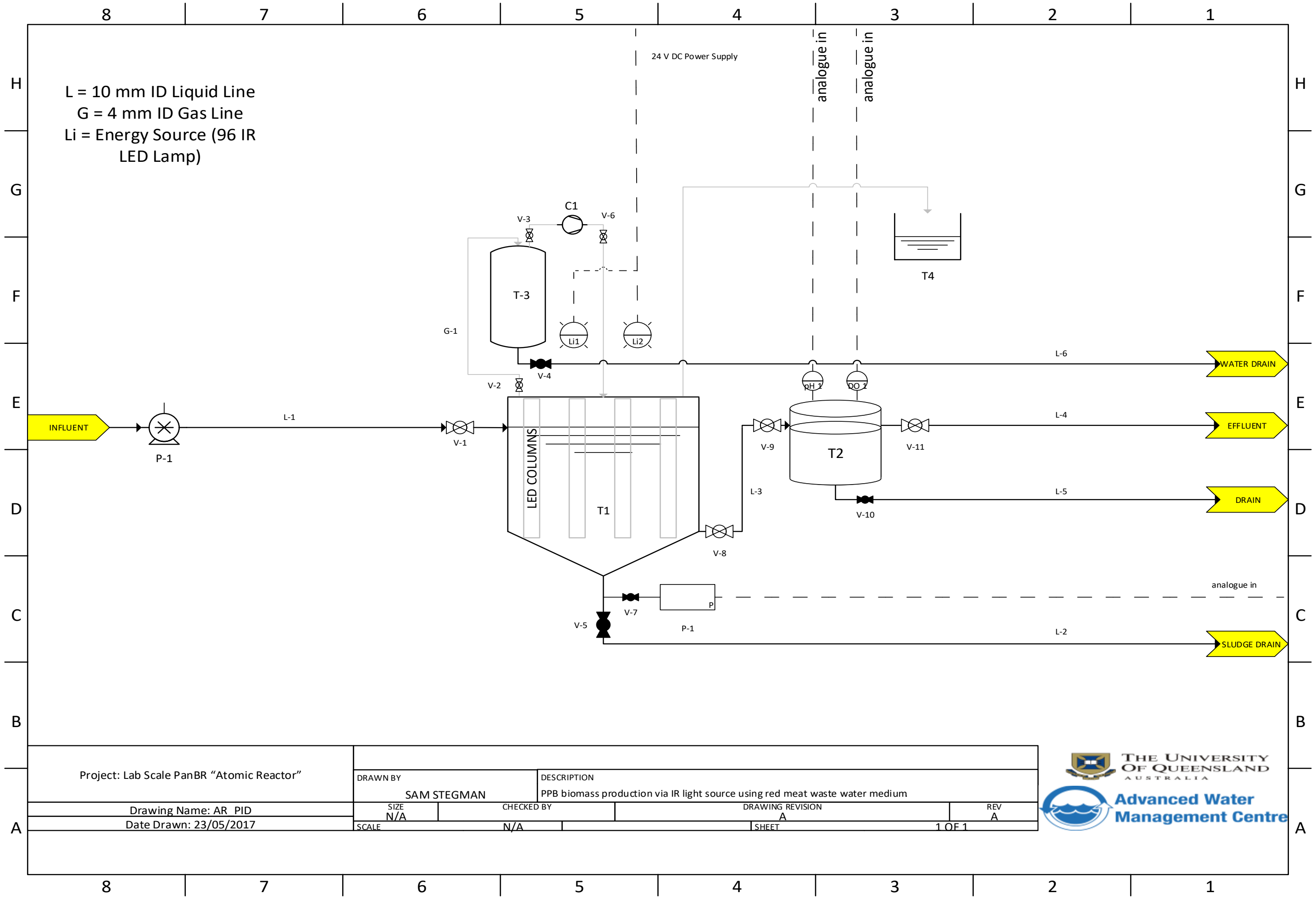


Figure 5: Attached growth photo bioreactor with PPB attached to submerged illuminated surfaces (tubes).

AMPC



Project: Lab Scale PanBR "Atomic Reactor"

Drawing Name: AR_PID

Date Drawn: 23/05/2017

DRAWN BY SAM STEGMAN

DESCRIPTION PPB biomass production via IR light source using red meat waste water medium

SIZE N/A

CHECKED BY

DRAWING REVISION A

REV A

SCALE N/A

SHEET 1 OF 1

Figure 6: Detailed schematic of attached growth reactor set-up with illuminated tubes.

3.5 Multi Chamber Photo Bioreactor

Multi Chamber Photo Bioreactors (MCPB) were developed as an alternative PPB process without the requirement for manual biofilm scraping. At small scale, such as laboratory or pilot scale, the multi-chamber rectors are more flexible as light can be provided from the side of the reactor to promote attached growth or from the top of the reactor to promote growth in the bulk liquid. In the multi-chamber reactors, there are multiple treatment steps. Each treatment step can be operated under identical conditions, or process conditions can be varied to optimize to achieve different process goals. For example, infra-red light could be supplied to the first chamber to promote PPB production, while visible light could be supplied to the final chamber to promote algae.

A 5L lab-scale attached growth reactor developed for this project is shown in Figure 7; a more detailed schematic of this reactor is shown in Figure 8. The multi chamber photo bioreactor contains 3 reactor chambers:

- Chamber 1 facilitates settling and hydrolysis of particulate material in the wastewater,
- Chamber 2 facilitates production of high quality PPB biomass,
- Chamber 3 is a polishing step.

The reactors are designed to allow separate performance assessments of each chamber and the required coupling of chambers to achieve sufficient treatment (in series).

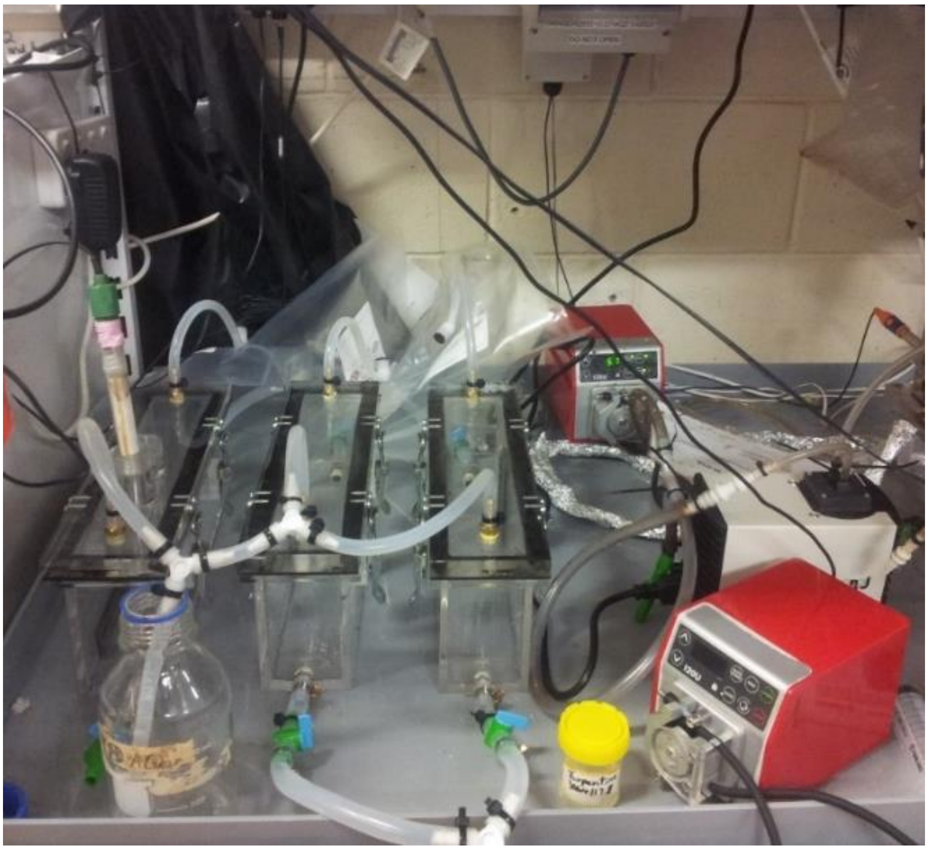
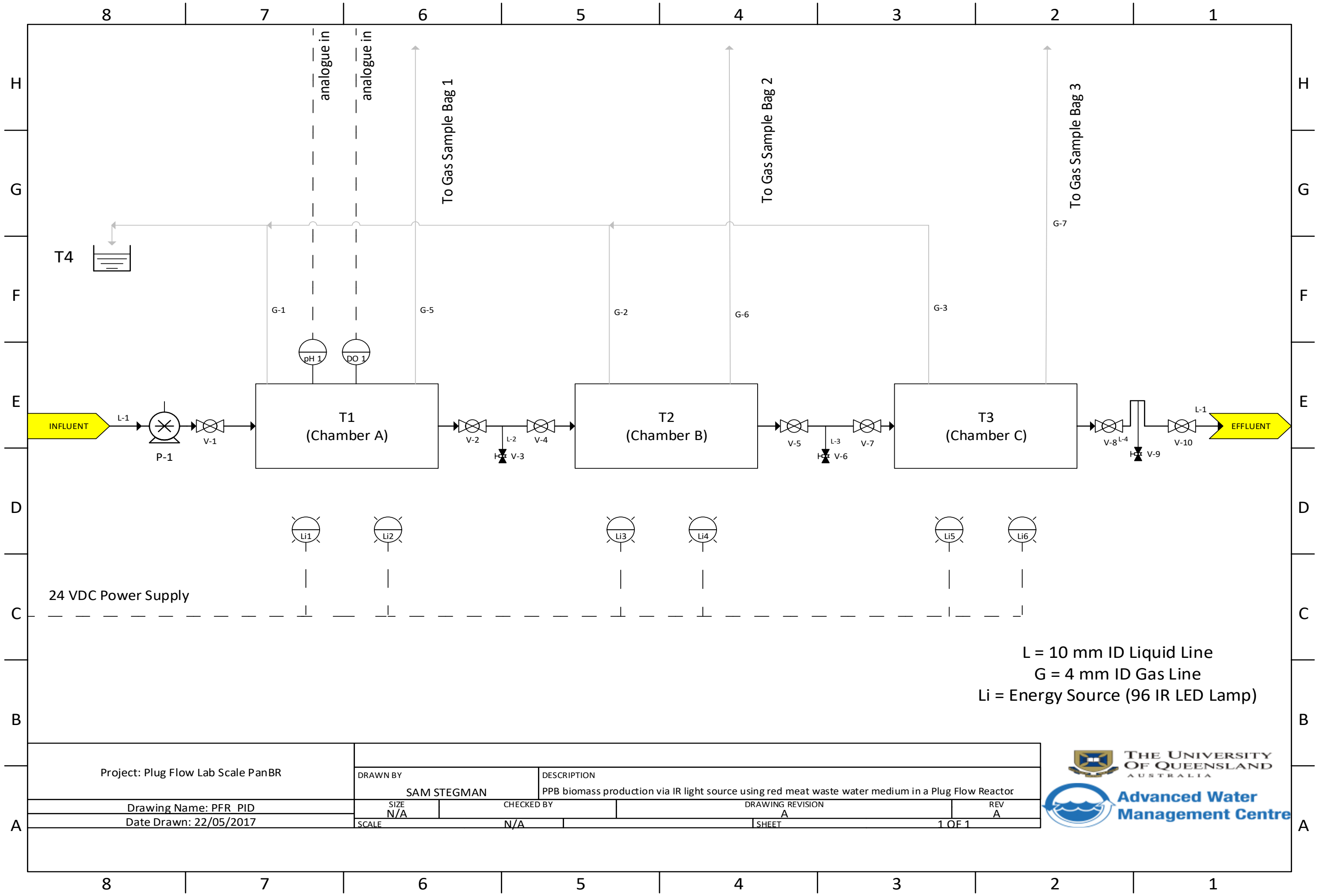


Figure 7: Mixed chamber photo bioreactor set-up.



L = 10 mm ID Liquid Line
G = 4 mm ID Gas Line
Li = Energy Source (96 IR LED Lamp)

Project: Plug Flow Lab Scale PanBR
Drawing Name: PFR_PID
Date Drawn: 22/05/2017
DRAWN BY: SAM STEGMAN
DESCRIPTION: PPB biomass production via IR light source using red meat waste water medium in a Plug Flow Reactor
SIZE: N/A
CHECKED BY
DRAWING REVISION: A
REV: A
SCALE: N/A
SHEET: 1 OF 1

Figure 8: Detailed schematic of mixed chamber photo bioreactor set-up.

3.6 Comparison of Reactor Designs

An initial comparison of reactor technologies is shown in Table 6. Reactors are compared based on estimated technology cost, footprint, PPB growth mode, product harvesting and product quality.

Currently, the primary differences between technologies are related to growth mode. We expect the attached growth biofilm formation to have greater potential for future application. Diffusion limitations and general substrate/biofilm contact may reduce the treatment rates in biofilm reactors compared to suspended growth reactors, However the major advantages include i) higher purity PPB biomass with higher protein content and ii) reduced moisture content in the harvested product without chemical addition or mechanical up-concentration and therefore reduced drying and processing costs. However, attached growth reactors require more complicated designs and more complicated reactor internals. This is expected to increase capital cost. Manual biomass harvesting/biofilm scraping regimes also require development. The combination of suspended growth and attached biomass harvesting in a hybrid design is a likely to merge the advantages of both systems but requires additional research.

At larger scale, the multi-chamber process could be implemented as a constructed bioreactor (using similar design principles to the lab reactors) or as a lagoon/raceway type system where a light filtering cover is used to supply the IR spectrum of sunlight. In this regard, the multi-chamber process is flexible enough to be implemented as either a higher-cost lower-footprint bioreactor option or a low-cost high-footprint lagoon option.

Table 6: Comparison of PPB Reactor Technologies

	Technology Cost	Footprint	PPB Growth Mode	Product Harvesting cost	Product Quality
PAnMBR	++	-	Suspended	++	+
Attached	+	-	Attached	-	-
Multi chamber	-	+ to ++	Suspended or Attached	+	+
Raceway	-	++	Suspended	++	++

(++) = high, (+) = moderate, (-) = low

4 RESULTS – PROTEIN FROM RED MEAT PROCESSING WASTEWATER

4.1 Red meat processing wastewater

Wastewater used in the project was collected from a cattle-only red meat processing (RMP) facility located in QLD Australia. The plant is a fully integrated slaughtering, fabricating, chilling, freezing and rendering facility that processes up to 6000 cattle per week. Red wastewater and green wastewater are transported separately within the RMP. Wastewater used in the project was red wastewater after primary treatment using rotating drum screen and dissolved air flotation (no chemical addition). After transporting, the sample wastewater was immediately placed in a fridge (-4°C) for storage and allowed to settle. The characteristics of the wastewater used in the project are presented in Table 7. The wastewater is relatively dilute compared to typical combined wastewater reported for Australian RMP (A.ENV.0131, AENV.0151).

Table 7: Characterisation of RMP wastewater used for SCP production.

Parameter	Concentration (mg/L)
TCOD	3266 ± 1841
SCOD	1335 ± 512
SCOD/TCOD	0.41
TS	2600 ± 1200
VS	1900 ± 1000
VFA (as COD equivalent)	293 ± 255
TKN	191 ± 57
NH_4	60 ± 28
TP	21 ± 6
PO_4	17 ± 6
TCOD/TKN/TP	**100/5.8/0.6**
SCOD/NH3/PO4	**100/4.5/1.3**
Al	0.02 ± 0.02
As	0.05 ± 0.05
B	0.06 ± 0.06
Ba	0.04 ± 0.01
Ca	21.8 ± 10.5
Cu	0.05 ± 0.02
Fe	1.7 ± 0.3
K	49.7 ± 21.0
Mg	17.4 ± 2.4
Mn	0.2 ± 0.06
Mo	0.01 ± 0.01
Na	128 ± 44
P	21.8 ± 7.1
Pb	0.10 ± 0.07
S	32.7 ± 14.5
Se	0.06 ± 0.06
Zn	0.04 ± 0.05

Note: Cd, Co, Cr, Mo, Ni were not detected during analysis of trace metals.

The TCOD/N/P ratio of the wastewater was 100/5.8/0.6, this suggests organics are present in excess and nutrients are more likely to limit PPB growth. Composition data also shows that approximately 40% of COD is solubilized and only 30% of nitrogen is present as ammonia. It is not clear if the remaining COD and nitrogen is in a form suitable for the PPB or if pre-fermentation is required.

4.2 Attached growth photo-bioreactor

4.2.1 Experimental Design

Lab-scale experiments were conducted in a 3 L attached growth reactor with IR illuminated submerged surfaces at ambient temperature (Section 3.4.3). As shown in Figure 9, the experimental setup included the illuminated (reaction) chamber, which can be operated in continuous or batch mode. Mixing was achieved by re-circulating the gas headspace through a sparge point at bottom of the reactor using an air-compressor. The reactor was covered with foil to control light exposure at all times. PPB attach to the outside of the tubes and form a biofilm which can be harvested through (1) a wiping system able to push the attached biomass towards the conic bottom of the reactor (from where it can be periodically collected), and (2) by removing the lid to scrape of the tubes manually. Attached growth enables light supply independent of the reactor biomass content and harvesting of almost pure PPB without undesired wastewater solids. This enables high purity PPB harvest with high protein contents, which is expected to increase the market value.

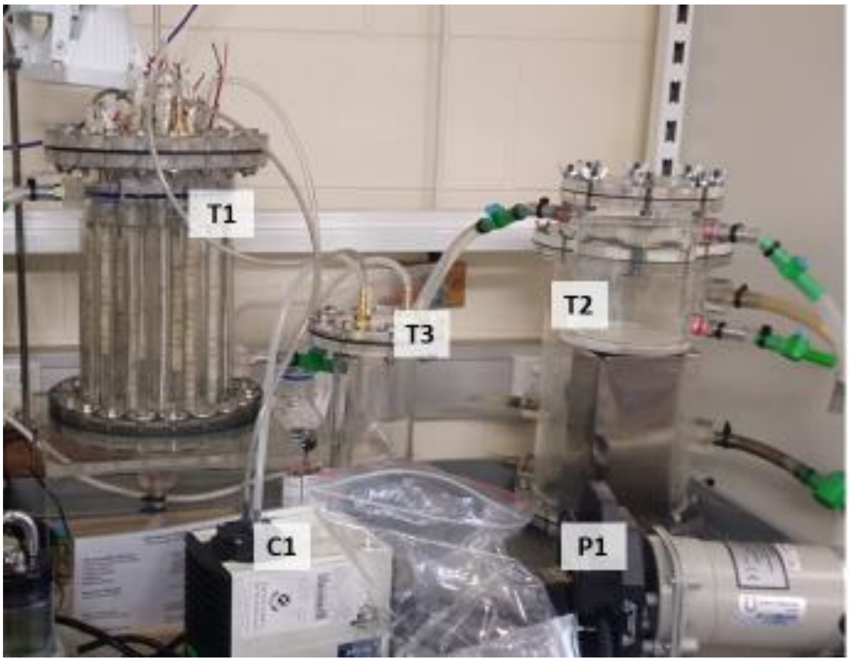


Figure 9: Attached growth schematic setup and laboratory configuration. (T1) photo-bioreactor; (T2) sampling reservoir; (T3) water trap (avoiding moist return towards the air compressor); (C1) air compressor for gas phase recirculation (from headspace to the bottom of the reactor); (P1) Feed pump.

At the start of operation (day zero) the reactor was inoculated with PPB previously grown on domestic wastewater. The operation of the reactor was set in different phases described in Table

8. During Phase 1 and 2, the reactor HRT was 2 days, resulting in an organic loading rate of ~1.6 gTCOD $L^{-1}d^{-1}$. During Phases 1 and 2 the light intensity was set to the maximum capacity provided by the power supply to promote maximum productivity rates in the system. Biomass harvesting was achieved via the wiping system at intervals corresponding to an SRT of 3-4 days. Biomass was kept in a freezer (-20°C) for further characterization with focus on the crude protein, amino acids content, chlorophyll and carotenoids.

During Phase 3, the gas recirculation was replaced with liquid recirculation and the reactor was operated in batch mode. During Phase 3, the light intensity was controlled at an average of 5.8 W m^{-2}. A graphical representation of the light distribution through the hollow tubes (in the presence of 0.5 g L^{-1} PPB biomass) in the attached growth system is presented in Figure 10.

Table 8: Operational phases of the attached growth photo-bioreactor.

Phase	Mode	HRT (days)	Loading (gCOD/L/d)	Description
1	Continuous	2	1.6	Enrichment / adaptation (continuous mode)
2	Continuous	2	1.6	Productivity determination (continuous mode) – harvest via wiping system
3a	Batch		N/A	Productivity determination (batch mode) – manual harvest; process optimization
3b	Batch	3	N/A	Productivity determination (batch mode) – manual harvest; process optimization
3c	Batch	2	N/A	Productivity determination (batch mode) – manual harvest; process optimization

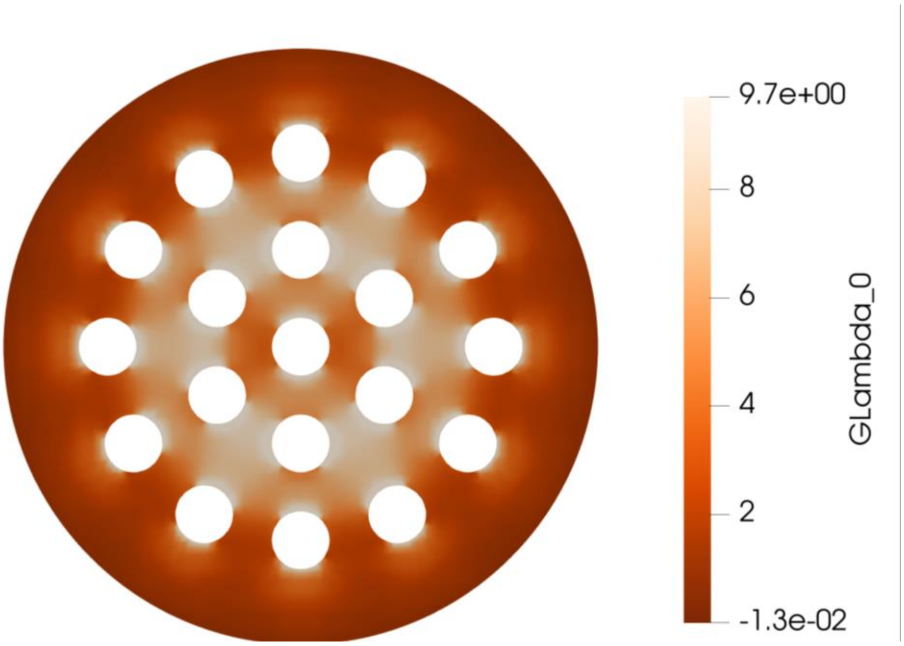


Figure 10: Graphical representation of light distribution throughout the hollow tubes in a cross-section of the attached growth setup. The presented data considers the concentration of 0.5 g L^{-1} PPB biomass within the liquid phase, meaning that the incident irradiance (5.8 W m^{-2}) is partly absorbed.

4.2.2 Process Results – Continuous Operation

During operation of the attached growth bioreactor, COD should be removed from the wastewater and converted to PPB biomass, if PPB growth is occurring as part of a biofilm both total COD and soluble COD removal rates should be high. Removal of total COD and soluble COD during continuous operation of the attached growth reactor is shown in Figure 11. Removal of total COD was generally above 50%, while removal of soluble COD was higher at >75%. This result is consistent with expectations that PPB readily metabolise soluble organic acids, but do not have the capacity to metabolise some forms of particulate organics. Alternatively, a portion of PPB may be growing in suspension and not in the biofilm, in this case a particulate COD may be converted to PPB biomass, but is lost in the effluent and not captured in the system.

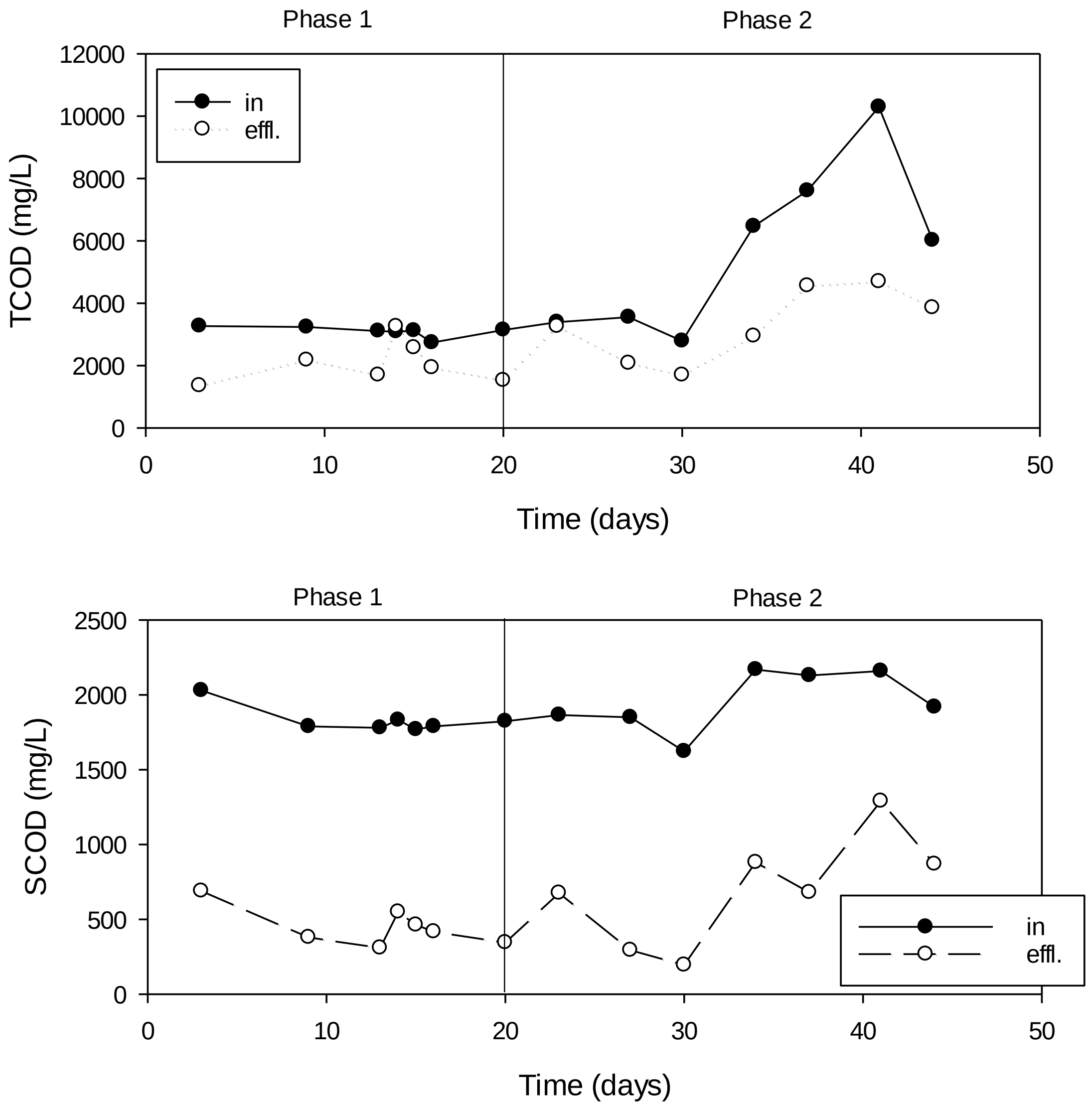


Figure 11: (A) Total COD and (B) Soluble COD removal during continuous operation of the lab-scale attached growth photo-bioreactor.

Samples of raw wastewater, effluent and harvested PPB are shown in Figure 12; the purple colour in the effluent suggests that PPB biomass is present in suspension, meaning that COD conversation rates were actually higher than the 50% COD removal recorded for the reactor.

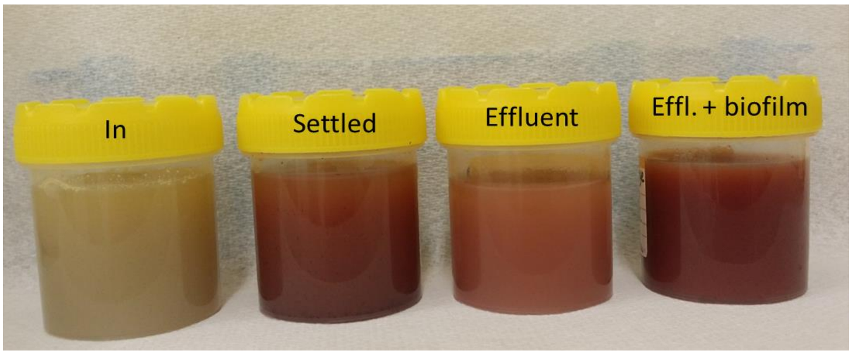


Figure 12: Samples of Influent (red meat processing wastewater), settled content (before re-suspension of attached biomass), liquid phase (equivalent to effluent content) and liquid + re-suspended biofilm content.

Figure 13 demonstrates the PPB biomass during initial stages of reactor operation, grown both in the liquid phase and attached to tubes. During continuous operation, areal productivity of the biofilm reached a maximum of 58.3 g COD m^{-2} d^{-1} (approximately 31.2 g VS m^{-2} d^{-1}) based on TCOD consumption. However, biomass recovered through harvesting was significantly lower at 18.4 g COD m^{-2} d^{-1} (corresponding to 9.8 g VS m^{-2} d^{-1}, concentrated at 50 g L^{-1}). Figure 13 (center and right) shows significant scum formation in the continuous attached growth bioreactors. Scum accumulation was largely the result of gas recirculation within the reactor and led to an overestimation of PPB productivity using the COD method.

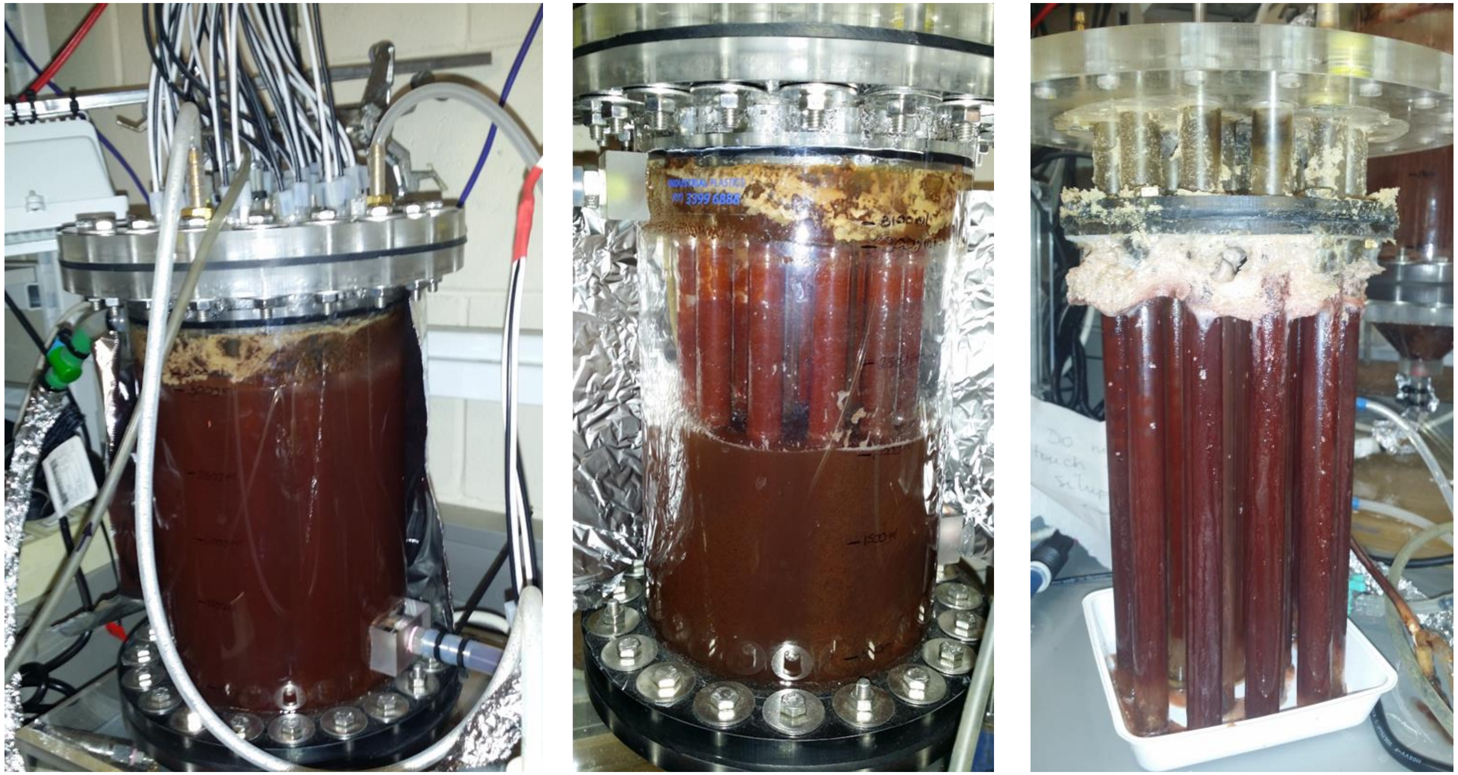


Figure 13: Grown PPB biomass in during continuous operation.

4.2.3 Process Results – Batch Operation

During Phase 3 the attached growth bioreactors were operated in semi continuous/batch mode. Batch times were 1 to 3 days. After each batch, 75% of the reactor volume was decanted and 25% was retained to inoculate the subsequent batch. This mode of operation reduced washout of PPB biomass in suspension (i.e. during harvesting) and improved effluent quality. The incident irradiance on the illuminated tubes (reaching the liquid phase) was controlled at an average of 5.8 W m^{-2} during batch 3 to promote consistent growth conditions.

During Phase 3A (Batches 1-3), COD was being removed at a maximum rate of 1.7 g COD L d^{-1} (Figure 14), however biofilms were not fully developed. Areal productivity during this period appeared very high based on COD balancing, however the recovered production was only 1.6 – 5.1 g TS m^{-2} d^{-1} (Figure 15). Poor agreement between the COD balances and the recovered PPB production was attributed to air intrusion through the air compressor and accumulation of particulate COD in the scum layer. As previously demonstrated (Huelsen *et al* 2016), COD availability can be an important limiting factor in the removal of N and P (thus in the growth of PPB biomass from wastewater).(Hülsen et al. 2016) Therefore, the gas recirculation mixing system was replaced by a liquid recirculation mixing system to minimise scum formation.

During Phase 3B (Batches 4-6, HRT 3 days), COD removal rates appeared significantly lower than during phase 3A, however there was no scum formation during this period and there was a much closer agreement between areal productivity measured via COD balancing and via direct biomass measurement. Areal productivity of recovered biomass was higher during this period at 4.1 to 7.8 g TS m^{-2} d^{-1} (Figure 15). VFA-COD was very limited at the beginning of batches in Phase 3B (<100 mg/L) and there was no accumulation of VFA during the batches, actually the mass of COD removed was higher than the VFA-COD fed to the reactors suggesting that all soluble COD and a portion of the particulate COD was converted to PPB. Similarly, total nitrogen removal was equal to or higher than nitrogen fed as NH_3 . The combination of these results supports the conclusion that all soluble components are converted to PPB, and that PPB production was limited by availability of the particulate compounds.

Shorter batch times were tested during Phase 3C (Batches 7-8, HRT 2 days) resulting in higher calculated productivities of 11.6 and 10.5 g TS m^{-2} d^{-1}. The achieved production rates are within the lower range of previously reported algae production systems.(Apel et al. 2017, Arbib et al. 2017, Lee et al. 2014, Min et al. 2014, Slade and Bauen 2013) Importantly, part of the formed biofilm (e.g. attached to reactor walls rather than on the tubes) could not be harvested at this stage, thus partly accounting for the differences between produced (attached) and recovered biomass. COD and nitrogen removal rates were similar or higher at the 2 day HRT when compared to the longer 3 day HRT in Phase 3B. The results indicates that the shorter batch times (2 days) are more than sufficent for complete uptake of the degradable COD. Therefore batch times may be reduced further, which will have the impact of further increasing areal productivity (i.e. similar biomass yields, but less growth time). However pre-processing is likely required to increase the portion of degradable COD and nitrogen in the wastewater.

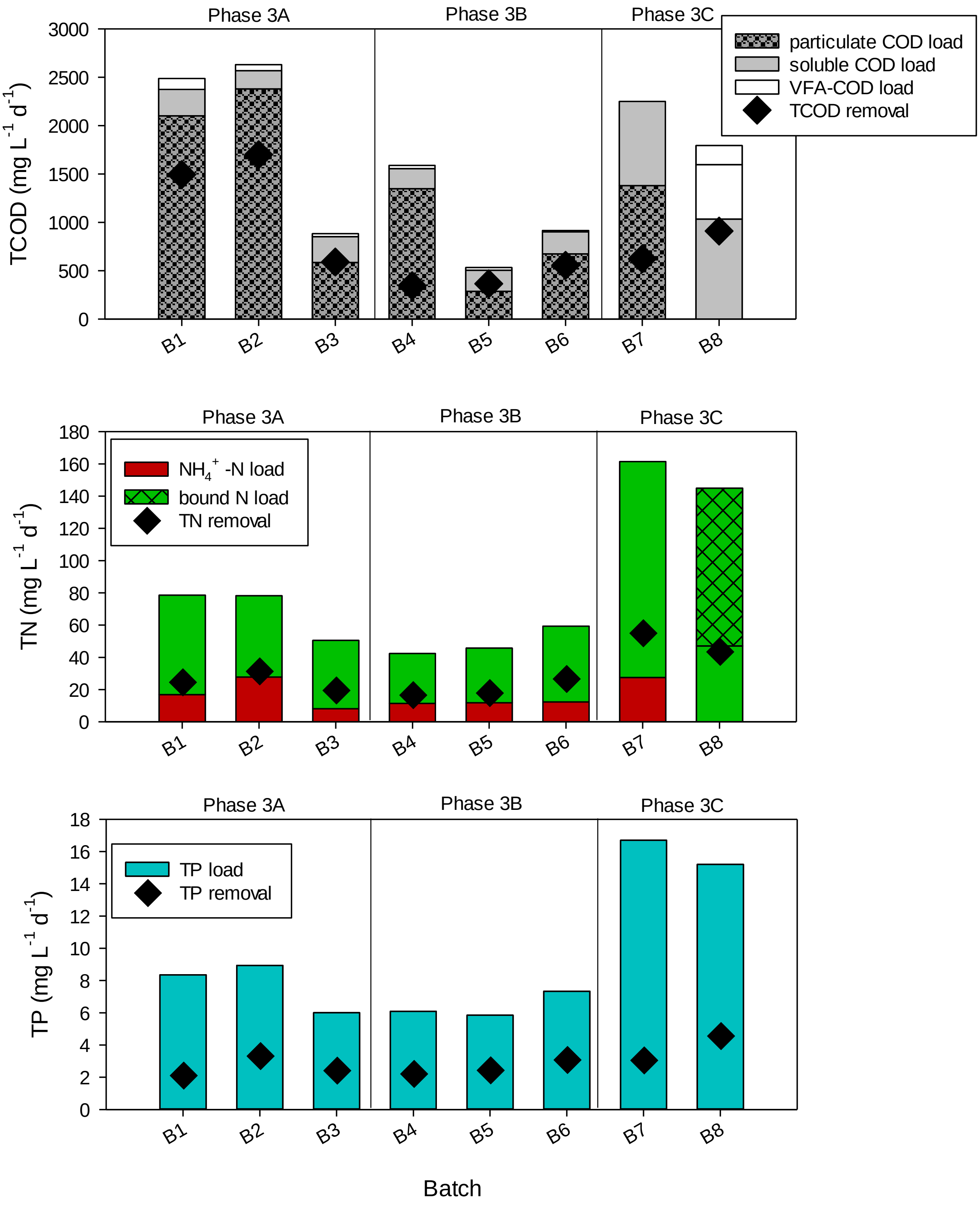


Figure 14: (a) particulate, soluble and VFA COD loading rate, and TCOD removal rates during Phase 3; (b) bound (protein) and NH_4^+-N loading rates and Total Nitrogen removal rates during Phase 3. (B1-B3, Phase 3A) Gas phase recirculation, 3 days HRT; (B4-B6, Phase 3B) Liquid phase recirculation, 3 days HRT; (B7-B8, Phase 3C) Liquid phase recirculation, 2 days HRT. Data of VFA-COD in B7 is not available.

Wastewater used in Phase 3C contained higher soluble COD and VFA content and higher soluble nutrients for immediate PPB uptake. Soluble nutrients further increased during the batch, however soluble COD and VFA were consumed. These results further support the conclusion that PPB growth was limited by COD availability during all batches, COD availability may be improved through pre-treatment or pre-fermentation.

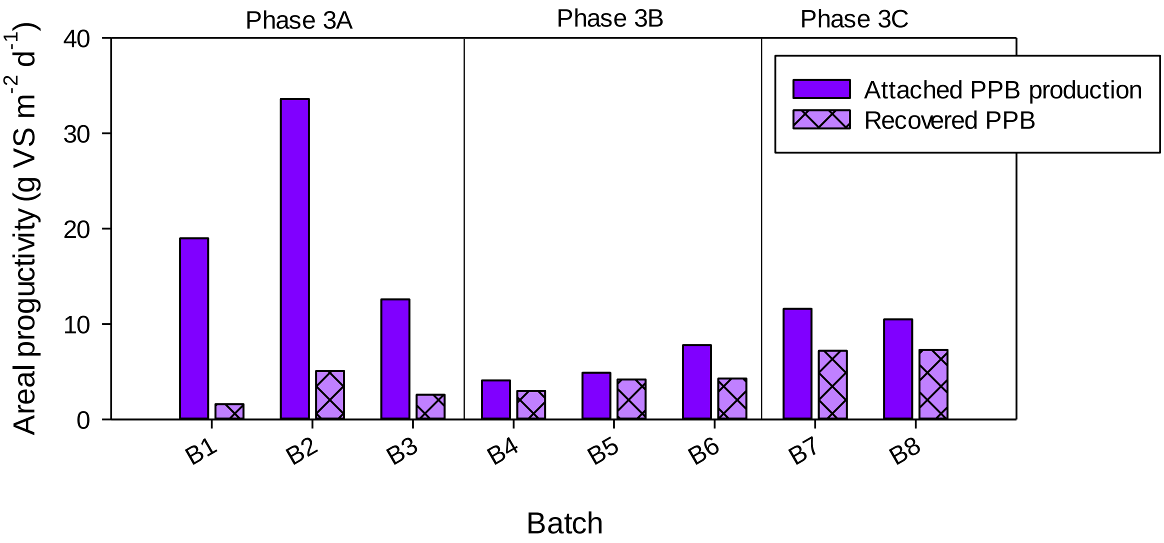


Figure 15: Calculated and recovered PPB biomass production during the batch operation (Phase 3). Attached PPB production is measured using COD balances. Recovered PPB production is via direct measurement of the biomass recovered.

4.2.4 Characterization of PPB biomass

PPB biomass harvested from the tubes during batches 1-8 contained approximately 98 (±33) gVS/L. Compared to conventional algal ponds the PPB biofilm biomass is harvested up to 100 more concentrated which is relevant for downstream processing as this can represent up to 30% of the overall algae production costs (LITT).

A detailed characterization of the harvested biomass is presented in Table 9, indicating a high nutrient and crude protein content, as well as high pigment content (determined as Total Carotenoids and Bacteriochlorophyll).

Carotenoid concentrations described in Table 9 are within the high range of previously reported PPB and microalgae values (3.5 – 13 g/kg PPB or algae biomass),(Liu et al. 2015, Liu et al. 2016, Saejung and Apaiwong 2015, Wang et al. 2017) higher concentrations may be detected through manipulating growth conditions or through further development of extraction methods. Similarly, the Bacteriochlorophyll concentrations detected within the attached PPB biofilm are slightly higher than those reported in the literature (1-18 g/kg VS).(Craggs et al. 2012, Liu et al. 2016, Wang et al. 2017).

Table 9: PPB biomass characterization in the attached growth setup (batch operation). The values are provided in average (standard deviation).

Parameter	Concentration		Fraction of VS	
TCOD	176 ± 60	(g/L)	1.79	(g/gVS)
VS	98 ± 33	(g/L)	-	
TN	10.8 ± 0.1	(g/L)	0.11 ± 0.01	(g/gVS)
TP	1.0 ± 0.05	(g/L)	0.01 ± 0.00	(g/gVS)
Protein	65 ± 5	(g/L)	0.66 ± 0.05	(g/gVS)
Carotenoids	930 ± 100	(mg/L)	9.5 ± 1.1	(mg/gVS)
Bacterioclorophyll	1,870 ± 100	(mg/L)	19.1 ± 0.9	(mg/gVS)

4.2.5 Operational Issues

Biomass harvesting was only moderately successful. While the wiping system was able to remove the biofilm effectively, movement of the wiper created an intense mixing effect that re-suspended the harvested biomass within the bulk liquid. This lead to an underestimation of the attached biomass collected via the reactor bottom. Therefore, during this Phase PPB productivity was assessed by completely re-suspending the biomass within the reactor water column, which was then quantified by analyzing the liquid phase before and after re-suspension (demonstrated in Figure 12).

At the end of Phase 2, compressor leaks resulted in oxygen intrusion (high dissolved oxygen), which affected the anaerobic PPB and the attachment on the tubes, thus considerably decreasing the productivity (data not shown).

Large amounts of scum were formed due to the high solid content of the wastewater feeding the reactor (Figure 13), compromising the mass balance and removal efficiency calculations and affecting bacterial attachment). Furthermore, we identified that the complete removal (harvest) of the biofilm from the tubes would affect the quality of the treated wastewater in continuous flow mode (as the PPB would require some time for re-growth), as demonstrated by the daily TCOD measurements highlighted in Figure 11. This can be rectified by interval harvesting of specific areas in a larger reactor.

4.3 Multi-chamber Photo Bioreactors

4.3.1 Experimental Design

Lab-scale experiments were conducted in a 5L multi chamber photo bioreactor. The set up contained 3 chambers (approx. 1.5L each) connected in series. The reactor operated at ambient temperature and reactor chambers were not actively mixed. Illumination was provided from above the reactor to promote suspended growth. At the start of operation (day zero) the reactor was inoculated with PPB previously grown on domestic wastewater. The multi chamber photo bioreactor was operated in three phases, as detailed in Table 10.

Reactor operation in Phase 1 focused on assessing wastewater treatment efficiency (COD, N and P removal), the reactors received no mixing during this phase resulting in (1) biomass stratification across the water column and (2) attached growth on the reactor walls. PPB biomass was not harvested during Phase 1. Reactor operation in Phase 2 focused on harvesting and characterizing PPB biomass. During Phase 2, the reactor contents were mixed intermittently to enable PPB sampling. The intermittent mixing impacted the removal efficiencies during Phase 2, as there was no sophisticated biomass retention system. Reactor operation in Phase 3 was similar to Phase 2, however the scum layer was sampled regularly and periodically removed as a biomass harvesting technique.

Table 10: Multi chamber photo bioreactor experimental phases

Phase	HRT	Notes
1	3 days each chamber	Enrichment, determination of removal efficiencies (COD, N and P)
2	3 days each chamber	Reactor content characterization
3	3 days each chamber	Product characterization (“scum” PPB)

4.3.2 Light Distribution

Figure 16 shows the distribution of the incident radiation on the lab scale mixed chamber setup (mean value of 15.8 W m^{-2}). The depth of the lab scale chambers (8 cm) is at the lower range of depths used in microalgae production ponds (7.5 – 35 cm).(Arbib et al. 2017, Craggs et al. 2012, Eustance et al. 2016) However, illumination is critical for growth of phototrophic single cell protein and light intensity will reduce below the water surface. Figure 17 shows the predicted transmissivity of light through the water column based on Murphy and Berberoglu.(Murphy and Berberoglu 2014) Figure 17 includes transmission of both visible light suitable for algae (430 nm) and IR light suitable for PPB (850 nm); and the impact of suspended biomass. Transmission of IR light is poor in comparison to visible light, and is heavily impacted by suspended biomass. At biomass concentrations of 0.1 g/L, 50% of light will be absorbed in the top 3-4cm of the lab-reactor with ~15% of light reaching the bottom of the chamber.

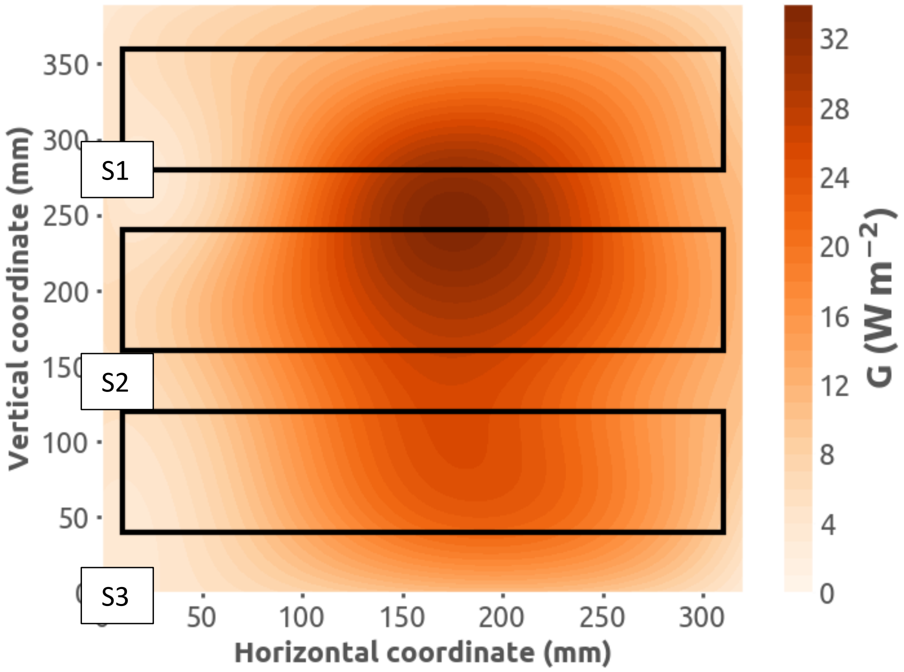


Figure 16: Incident radiation on the mix chamber setup. (S1-S3) Chambers 1-3.

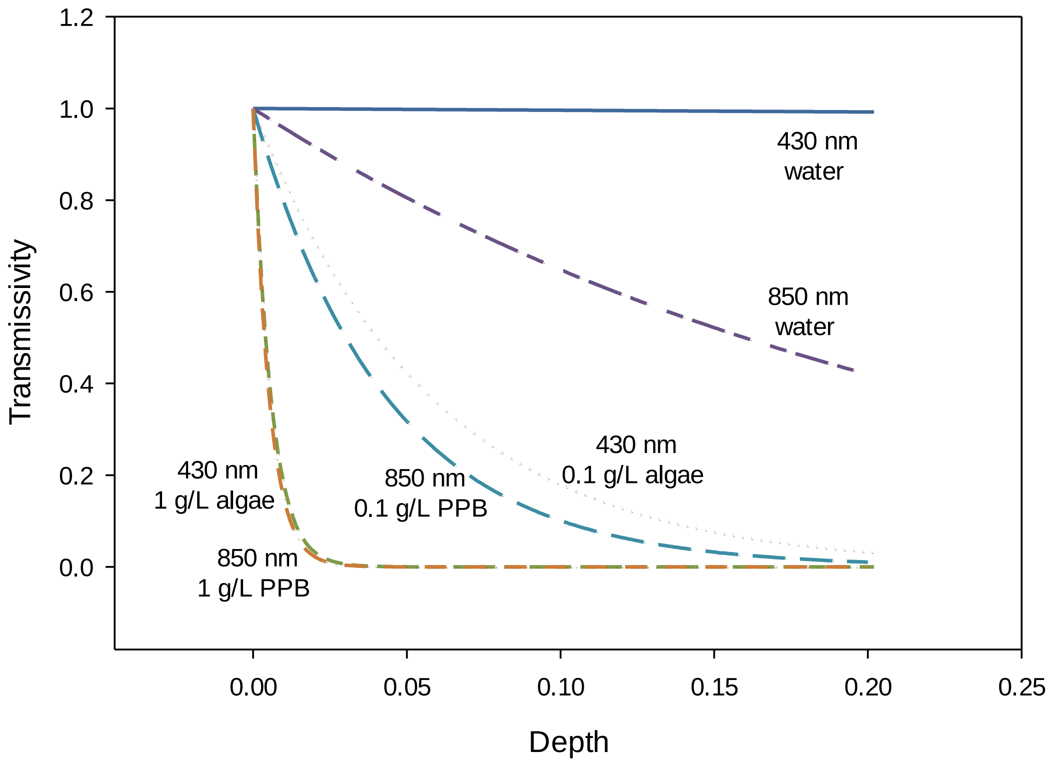


Figure 17: Transmissivity of 430 and 850 nm wavelength irradiations in water, in the absence and presence of microorganisms (algae and PPB) at 0.1 and 1 g VS/L.

4.3.3 Process Performance

Figure 18 shows the concentrations of soluble COD in the raw wastewater and effluent from each chamber and the calculated removal efficiencies. Soluble COD was removed at all times during process operation. The removal rates in Phase 1 (Days 0 – 39) averaged 69.3 ±5.5% in chamber 1, which was further increased to 81.8 ±5 % and 88.5 ±5 % after Chambers 2 and 3 respectively. This indicates the SCOD is effectively converted into PPB biomass which can potentially be separated from the liquid phase by settling. Figure 19 shows the concentrations of Total COD in the raw wastewater and effluent from each chamber and the calculated removal efficiencies. Removal of TCOD occurred in each reactor chamber. During Phase 1, an average of 52 ±12 % TCOD removal was achieved in Chamber 1. The overall removal efficiency was then increased to 72 ±11 % after Chamber 2 and finally achieved 92 ±12 % TCOD removal in Chamber 3. The results suggest growth and settling of PPB biomass is most effective in Chamber 1, however Chamber 2 and Chamber 3 contribute to wastewater polishing.

During Phase 2, settled biomass in the Chambers was frequently re-suspended to allow harvesting and characterization of the PPB biomass. Therefore net removal of TCOD was not expected during this period. However, the soluble COD removal was comparable to Phase 1 at 50.6 ± 7 %, 68.7 ±7 % and 81.2 ±5 % after Chambers 1, 2 and 3 respectively. Therefore, PPB growth was maintained and effective during Phase 2. The variable effluent TCOD in Phase 2 is a result of the resuspension process where accumulated solids were suspended and not able to resettle prior to being wash out. A sludge retention system could be added to prevent wash out of the biomass.

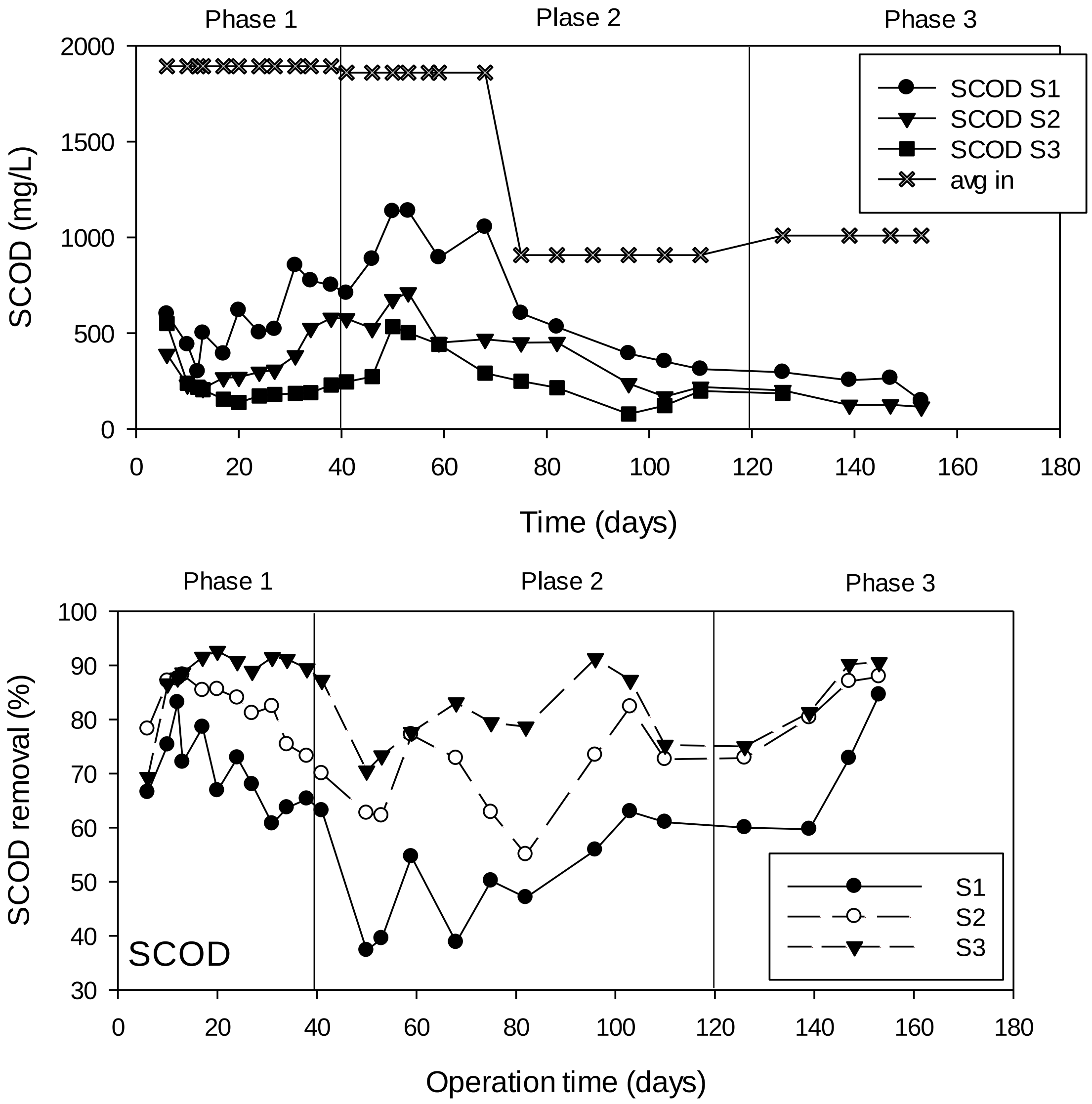


Figure 18: Process performance based on Soluble COD concentrations (Top) and Soluble COD removal efficiencies (bottom), considering influent wastewater (average) and effluent from chambers 1, 2 and 3.

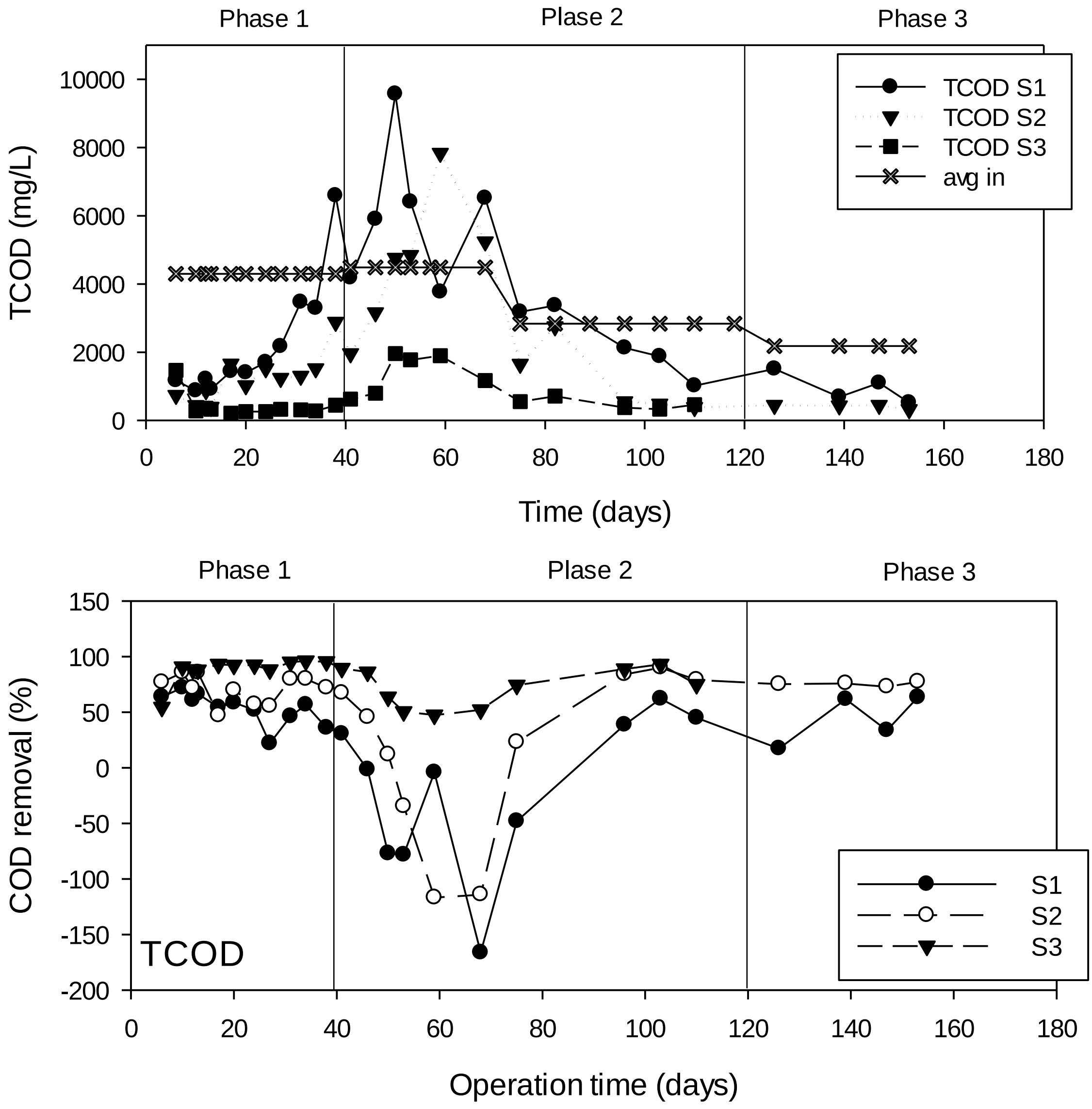


Figure 19: Process performance based on TCOD concentrations (Top) and TCOD removal efficiencies (bottom), considering influent wastewater (average) and effluent from chambers 1, 2 and 3.

VFA results presented in Figure 20 confirm that all readily degradable organics were consumed in the multi chamber photo bioreactor. The VFA concentration transferred from Chamber 2 to Chamber 3 was very low. Readily degradable COD, such as VFA can be efficiently transformed into PPB biomass, whereas other fractions require hydrolysis and fermentation (rate limiting) to become bioavailable for PPBs. Hydrolysis and fermentation requires more time and growth of a second community of fermenting bacteria in addition to PPB. The low concentration of VFA transferred to Chamber 3 indicates that the PPB growth in this chamber was limited by the available organics.

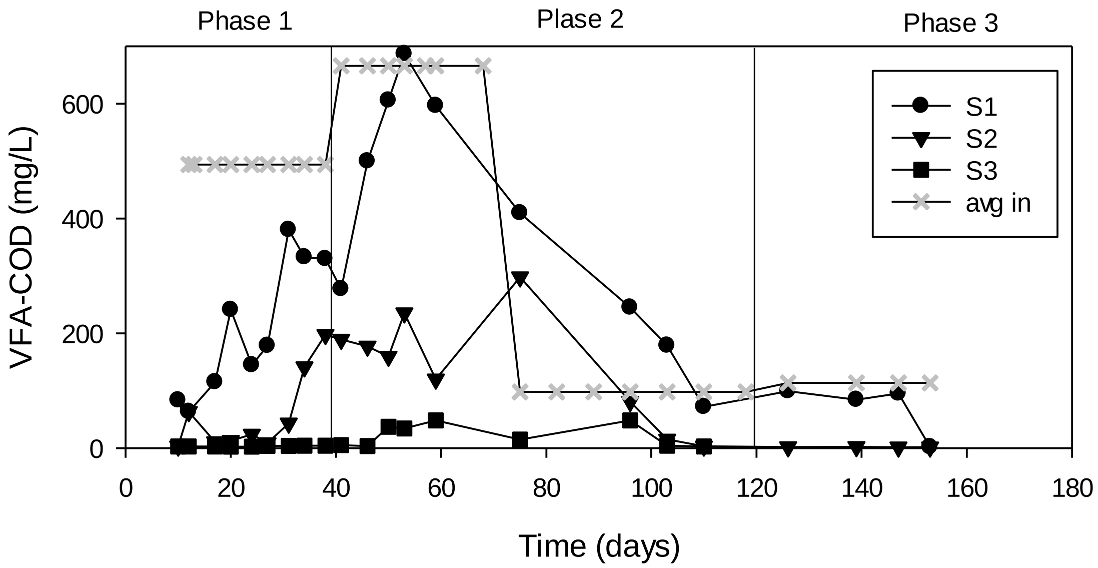


Figure 20: VFA-COD concentration of effluent from chambers 1-3.

Figure 21 shows the concentration of nitrogen and the nitrogen removal efficiency in the multi chamber photo bioreactor. Removal of total nitrogen in a PPB process occurs through settling of feed solids or through growth and capture of PPB biomass. Total nitrogen removal was relatively consistent at 50% during Phase 1, however there was some variability in removal from each Chamber. During Phase 2, total nitrogen removal was more variable, this also occurred with TCOD results and is consistent with biomass resuspension procedures and wash out of accumulated PPB biomass. Figure 22 shows the concentration of phosphorous and the phosphorous removal efficiency in the multi chamber photo bioreactor. Phosphorous data followed similar trends to nitrogen data and demonstrates that PPB growth was not phosphorous limited. During all stages of operation, there was an increase in ammonia in all reactor chambers. The increase in ammonia indicates that particulate organics and protein in the raw wastewater feed were hydrolyzed and released during the process. The presence of ammonia confirms that the PPB growth was not nitrogen limited and higher PPB yields can be achieved with higher degradable COD.

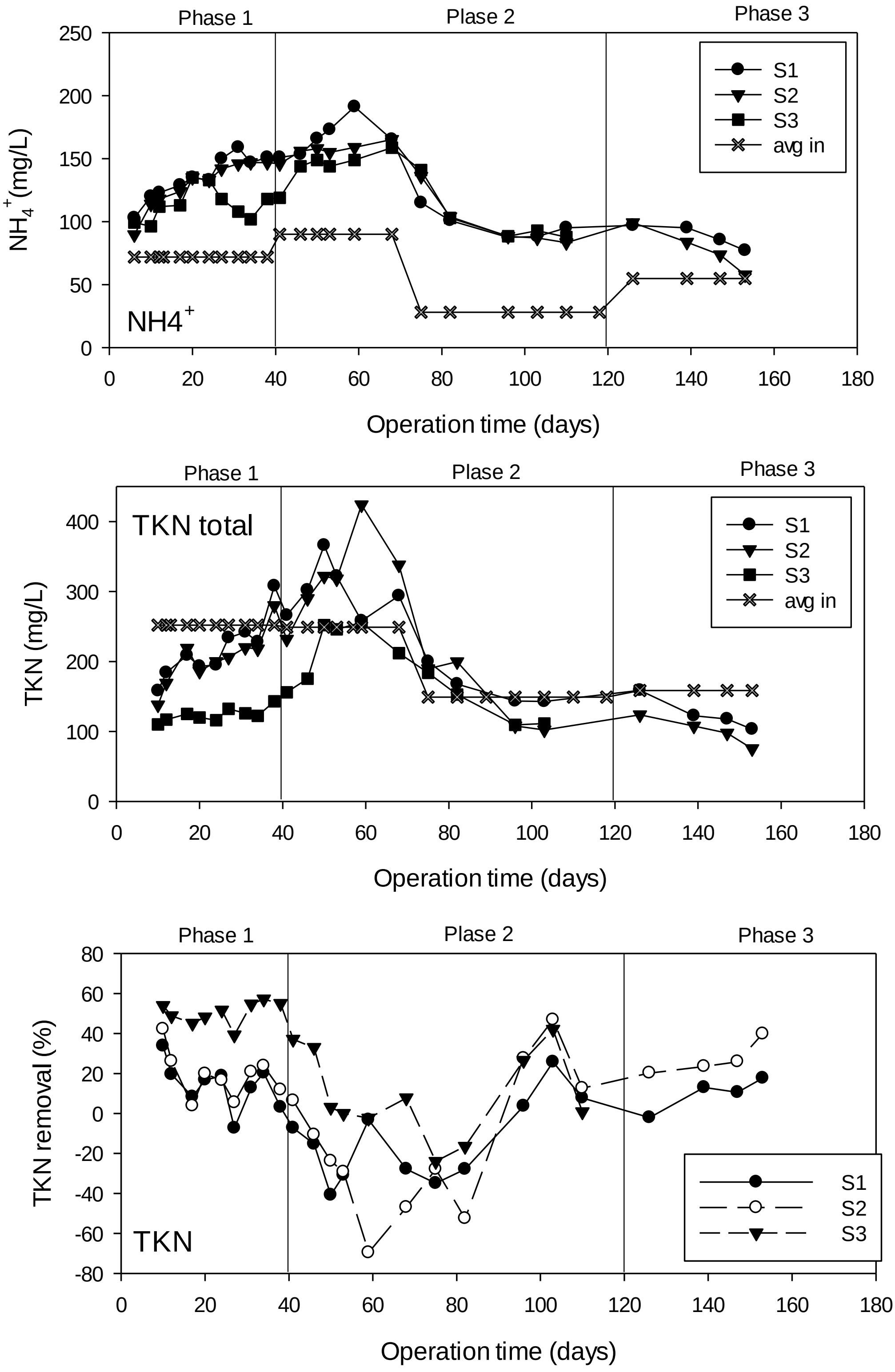


Figure 21: Process performance based on ammonia concentrations (Top), total nitrogen concentrations (middle) and nitrogen removal efficiencies (bottom), considering influent wastewater (average) and effluent from chambers 1, 2 and 3.

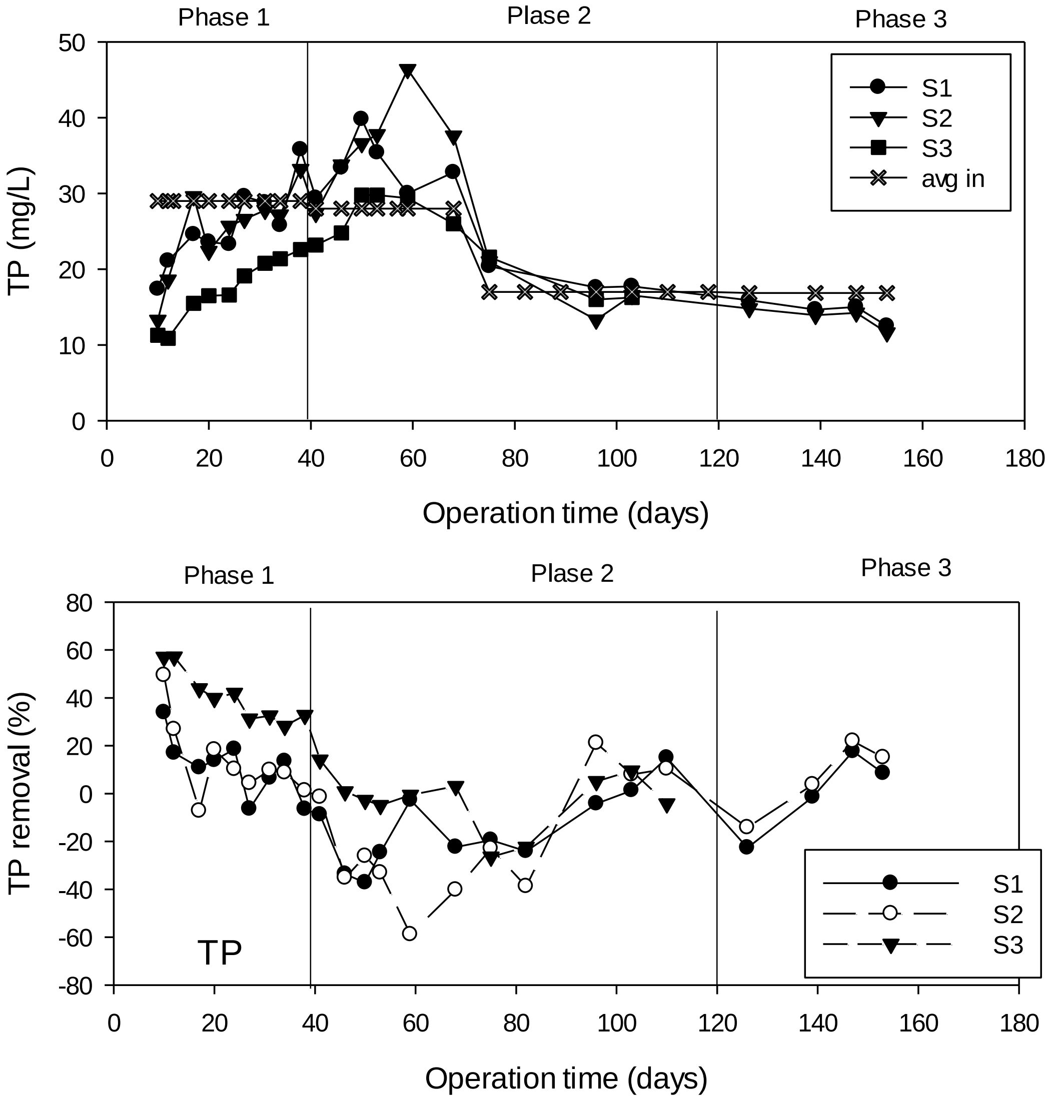


Figure 22 – Process performance based on phosphorous concentrations (Top) and Phosphorous removal efficiencies (bottom), considering influent wastewater (average) and effluent from chambers 1, 2 and 3.

4.3.4 Characterization of PPB biomass

Areal productivity during operation of the Multi Chamber Photo Bioreactor is shown in Table 11. Average productivity of PPB biomass was highest in Chamber 1, reaching 28.5 gVS m^{-2} d^{-1} during Phase 1. This productivity is at the higher range of values reported for algae systems (3.5 - 32 g VS m^{-2} d^{-1})$^{\text{(Apel et al. 2017, Arbib et al. 2017, Eustance et al. 2016, Tang and Hu 2016)}}$, however the protein concentration of PPB produced during this period was relatively low (30%). It is possible that particulate solids in the feed settled with the PPB biomass collected in Chamber 1, leading to an overestimate of PPB growth.

The suspended solids concentration in the multi chamber photo bioreactor varied between 3 and 12 g/L (i.e. Chamber 1, Phase 2), at these concentrations light transmission is expected to be very poor (Figure 17, Section 4.3.2). Therefore, the use of deeper ponds is likely not to improve the PPB productivity unless efficient mixing is provided.

Table 11: Average areal PPB biomass productivity in chambers 1, 2 and 3, during operational Phases 1 and 2.

	Biomass productivity (gVS $m^{-2}d^{-1}$)		
	Chamber 1	**Chamber 2**	**Chamber 3**
Phase 1	28.5 ± 12	14.1 ± 13.5	14.4 ± 10.8
Phase 2	17.9 ± 8.6	13.8 ± 3	4.1 ± 4.7

A detailed characterization of PPB biomass harvested from the multi chamber photo bioreactor is presented in Table 12. Samples for amino acid analysis are currently being prepared/submitted for determination. The biomass compositions varied between chambers with the lowest protein (30%), carotenoid (6.2 mg/gVS) and bacteriochlorophyll (12.4 mg/gVS) concentrations recorded in Chamber 1 and increasing in Chamber 2 and again in Chamber 3. The concentrations of proteins, carotenoids and bacteriochlorophylls in PPB biomass from Chambers 2 and 3 were high in comparison to literature and similar to the quality of PPB biomass collected from attached growth. The composition results support the conclusion that particulate organics in the feed settled with the PPB biomass in Chamber 1 with the amount of settled particulate contaminants decreasing in Chambers 2 and 3.

Table 12: PPB biomass characterization in the mixed chamber setup (Phase 2). Average values are provided, errors represent standard deviation.

Parameter	**Chamber 1**	**Chamber 2**	**Chamber 3**
TCOD	2.2 ± 0.3 (g/gVS)	2.3 ± 0.4 (g/gVS)	1.8 ± 0.2 (g/gVS)
VS	-	-	-
TN	0.05 ± 0.01 (g/gVS)	0.07 ± 0.01 (g/gVS)	0.10 ± 0.01 (g/gVS)
TP	0.005 ± 0.001 (g/gVS)	0.008 ± 0.001 (g/gVS)	0.011 ± 0.002 (g/gVS)
Protein	0.3 ± 0.1 (g/gVS)	0.6 ± 0.2 (g/gVS)	0.7 ± 0.2 (g/gVS)
Carotenoids	6.2 ± 0.9 (mg/gVS)	11.5 ± 0.6 (mg/gVS)	14.0 ± 2.0 (mg/gVS)
Bacterioclorophyll	12.4 ± 1.8 (mg/gVS)	23.4 ± 1.7 (mg/gVS)	27.1 ± 2.5 (mg/gVS)

Examples of influent and effluent samples from the multi chamber photo bioreactor are shown in Figure 23. The colouring of effluent from Chamber 1 supports the conclusion that feed particulates are present in this chamber, while the deep red colour in Chamber 2 is consistent with a higher quality PPB product. Chamber 3 is relatively dilute, consistent with conclusions that is chamber is more of a polishing step.

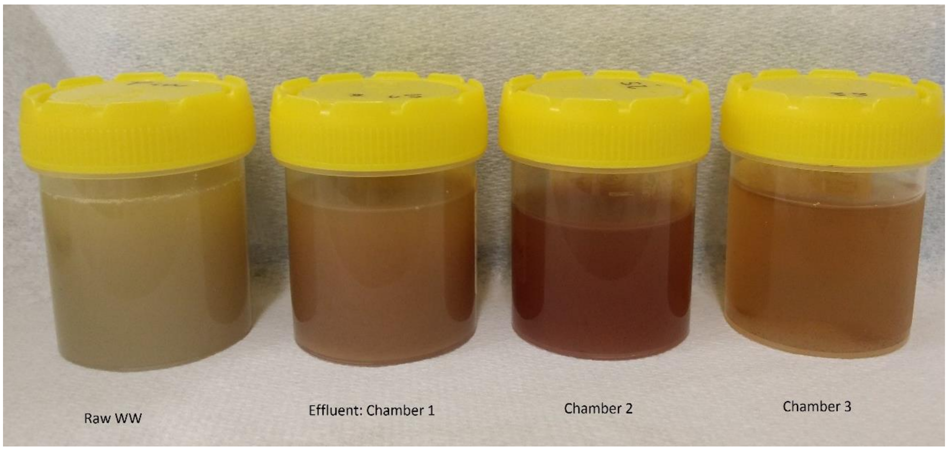


Figure 23: Influent wastewater and effluent samples from the series of chambers 1-3 during phase 1.

4.3.5 **Operational Issues**

Scum formation was a challenge during operation of the multi chamber photo bioreactor (shown in Figure 24). Scum formation was most prevalent in Chamber 1 and may have been partly due to fat, oil and grease in the raw wastewater feed. The presence of a concentrated scum layer on the chamber surface significantly reduces light transmission into the chamber and is therefore a significant operational challenge. However, if scum can be scraped from the surface of the chamber, this may represent a cost effective harvesting method.

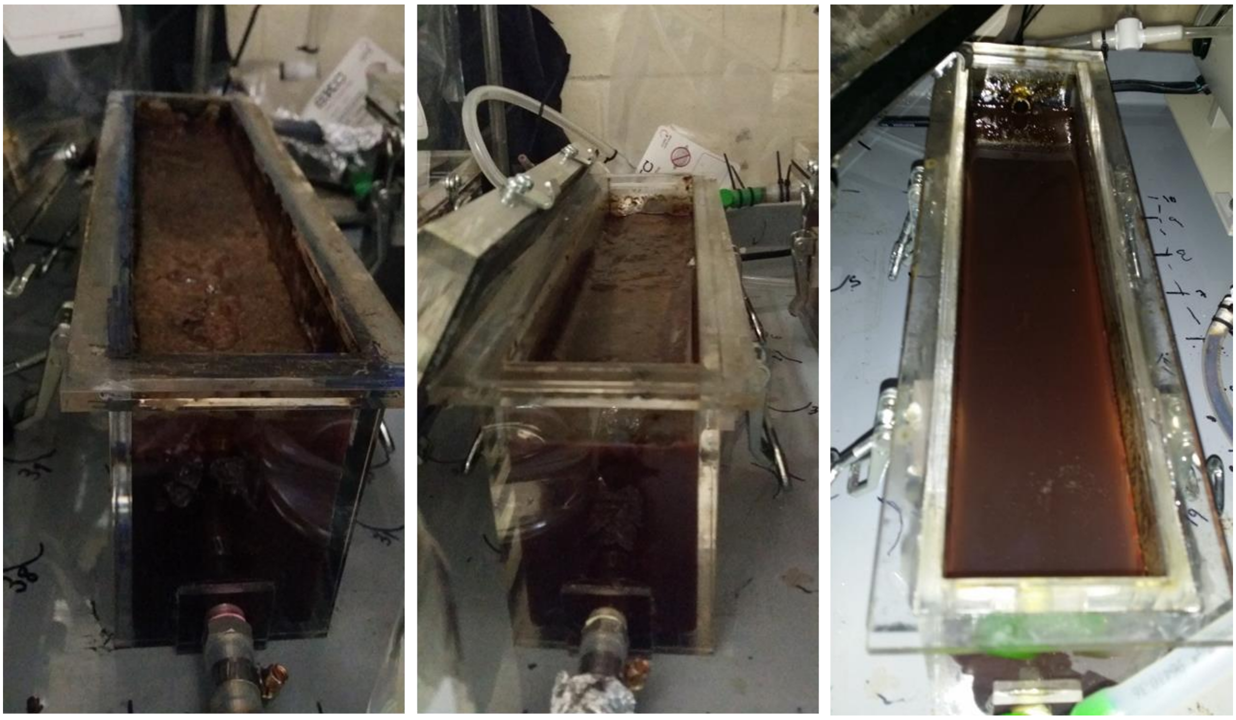


Figure 24: Phase 2: Chamber 1-3, indicating scum formation especially in Chamber 1.

4.4 Summary of PPB Performance Progress

Attached growth bioreactors and multi chamber photo bioreactors were constructed and operated at lab-scale with the following outcomes:

Attached growth photo bioreactor

- PPB can be grown using biofilms attached to submerged (IR) irradiated surfaces. The reactor design is complicated, however the biomass product is high quality and concentrated.
- Areal productivity rates of approximately 10gVS m^{-2} d^{-1} have been achieved during initial testing, these productivity rates are within the range reported for large algae systems and can be improved through optimisation.
- PPB biofilms can be harvested by scraping the surface of the illuminated tubes, however harvesting may be labour intensive and biomass recovery was limited at approximately 60% of PPB produced.
- Biofilm concentrations are approximately 100 gVS L^{-1} when recovered and represent a 100x increase in the concentration of suspended biomass in algae processes. This may significantly reduce harvesting and dewatering costs.
- PPB biomass contained a higher protein fraction (60%), which may represent high suitability as a feed additive.
- PPB biomass contained high concentrations of pigments that may represent a high value alternative to feed.
- PPB biomass was not impacted by variations in wastewater composition or quality. Product consistency is critical for end use as a feed or feed supplement.
- Conversation and capture of organics, nitrogen and phosphorous as PPB biomass was relatively low. PPB production was limited by readily degradable COD and may be significantly improved using a pretreatment or prefermentation step.

Multi chamber photo bioreactor

- PPB can grow in suspension using IR irradiation provided from above the reactor. Reactor designs are much simpler and product quality can be high. Product is generally not concentrated.
- Multi chamber reactors can be used to partially separate pre-hydrolysis, PPB growth and wastewater polishing, however the configuration of each step in not yet optimised.
- Areal productivity rates of 20-30gVS m^{-2} d^{-1} have been achieved in the pre-hydrolysis reactor, however the product quality was low and included settled particulate contaminants from the feed wastewater.
- Areal productivity rates of approximately 14gVS m^{-2} d^{-1} have been achieved in the PPB growth chamber. Very high quality with very low contamination was achieved in this chamber, however product quality was variable.
- PPB biomass concentrations were generally low in the suspension (3-12g/L) and require significant dewatering to up concentrate and dry.

- Overall, PPB biomass quality varied between compartments with low quality (30% protein) and high quality (60-70% protein) streams available.
- PPB biomass contained high concentrations of pigments which may represent a high value alternative to feed.
- PPB biomass was partially impacted by variations in wastewater composition or quality. Product consistency is critical for end use as a feed or feed supplement.
- Conversation and capture of organics, nitrogen and phosphorous as PPB biomass was relatively low. PPB production was limited by readily degradable COD and may be significantly improved using a pretreatment or prefermentation step.

PPB production from RMP wastewater was successfully achieved using both attached growth modes (such as biofilms) and using suspended growth modes. Attached growth resulted in a relatively consistent PPB product with high protein content (approx. 65%). Suspended growth resulted in more variable PPB product quality between 30% and 70% crude protein, this is partly attributed to the capture of wastewater particles in the product, which essentially dilute the product quality. While PPB product quality using is attached growth is higher and more consistent when compared to suspended growth, the attached growth reactors are more complicated and manual biofilm harvesting may be labour intensive. At larger scale, the suspended growth process could be implemented as a constructed bioreactor (using similar design principles to the lab reactors) or as a lagoon/raceway type system where a light filtering cover is used to supply the IR spectrum of sunlight. In this regard, the multi-chamber process is flexible enough to be implemented as either a higher-cost lower-footprint bioreactor option or a low-cost high-footprint lagoon option.

With either technology, capture of nitrogen in the PPB product and subsequent conversion to microbial protein was limited, largely attributed to the form of nitrogen entering the reactors. The research focus is now optimizing PPB yields, through the use of pre-fermentation and simplifying reactor design and operation for scale up the technology.

5 RESULTS – PROTEIN FROM RED MEAT PROCESSING SOLID WASTE

5.1 Concept

Australian slaughterhouses have the potential to generate large volumes of solid waste, originating in a number of processing areas with key sources including paunch, manure, screenings (not rendered), DAF sludge, aerobic wastewater sludge, contaminated cardboard and condemned/dead animals. Cattle paunch in particular is a major waste produced at cattle slaughterhouses and is comprised of partially digested cattle feed, mainly containing grass and grain. The volume and composition of paunch waste varies according to individual animals and site handling practices but is reported at approximately 60 kg of wet paunch waste per animal (5-7 kg solids), corresponding to approximately 10% of the total weight of the live animal.

Mushroom fermentation is an emerging technology that has been investigated for application to municipal solid waste. Potential advantages of mushroom fermentation include:

- Production of a relatively cheap source of high-quality food protein using degradable cellulosic wastes
- Production of nutritionally enhanced dietary supplements/mushroom nutraceuticals (Mushroom Biotechnology),
- Bioconversion of difficult lignocellulosic materials into highly degradable bioenergy feedstocks
- Bioconversion/bioremediation of environmental contaminants (i.e. heavy metals).

5.2 Red meat processing Solid Waste

Solid Waste used in the project was collected from a cattle-only red meat processing (RMP) facility located in QLD Australia. The plant is a fully integrated slaughtering, fabricating, chilling, freezing and rendering facility that processes up to 6000 cattle per week. Red wastewater and green wastewater are transported separately within the RMP. Solid waste used in the project was solid cattle paunch after primary treatment using a rotating drum screen. After transporting, the paunch waste was immediately placed in a fridge (-4°C) for storage and allowed to settle. The characteristics of the paunch solids used in the project are presented in Table 13. Additional solid waste streams are available at this RMP, but have not been tested during this project.

The COD:N:P ratio of the paunch solid waste was 100:1:0.3, the optimal ratio for PPB is 100:10:2, suggesting that paunch solid waste does not contain sufficient nutrients for a high yield waste-to-protein technology such as PPB. Additionally, the paunch solid waste contained >95% particulate organic material, and this material is unlikely to be metabolized by PPB in the particulate form. However, the high solids content and high carbon content of paunch solid waste may be suitable for alternative waste-to-value technologies such as mushroom fermentation.

Table 13: Characterisation of RMP solid waste used for SCP production

	Units	Paunch
TCOD	g.kg^{-1}	295 ± 18
sCOD	g.kg^{-1}	6 ± 5
TS	g.kg^{-1}	221 ± 26
VS	g.kg^{-1}	206 ± 24
VS/TS		0.94 ± 0.01
TCOD/VS		1.4 ± 0.1
Partial Alk. (pH 5.7)	mg CaCO$_3$.L^{-1}	1995
Total Alk. (pH 4.3)	mg CaCO$_3$.L^{-1}	3094
TKN	mg.kg^{-1}	3164 ± 683
NH3	mg.kg^{-1}	150 ± 64
TP	mg.kg^{-1}	916 ± 104
PO4	mg.kg^{-1}	619 ± 134
Al	mg.kg^{-1}	14.2 ± 12.5
B	mg.kg^{-1}	0.2 ± 0.3
Ba	mg.kg^{-1}	3.7 ± 1.3
Ca	mg.kg^{-1}	1049 ± 78
Cr	mg.kg^{-1}	0.1 ± 0.2
Cu	mg.kg^{-1}	5.1 ± 1.5
Fe	mg.kg^{-1}	62 ± 23
K	mg.kg^{-1}	940 ± 225
Mg	mg.kg^{-1}	233 ± 58
Mn	mg.kg^{-1}	18.3 ± 9.8
Mo	mg.kg^{-1}	0.8 ± 0.6
Na	mg.kg^{-1}	3176 ± 1514
Ni	mg.kg^{-1}	0.0 ± 0.0
P	mg.kg^{-1}	975 ± 230
Pb	mg.kg^{-1}	3.1 ± 2.6
S	mg.kg^{-1}	394 ±120
Zn	mg.kg^{-1}	48.5 ± 18.2

5.3 Batch Laboratory Experiments

5.3.1 Paunch with sterilization Pre-treatment

For initial tests, dewatered paunch was sterilized using autoclave treatment at 121°C. The sterilization pre-treatment was design to remove native rumen microbes and prevent completion for mushroom growth. Batch laboratory experiments were then conducted using pre-sterilized dewatered paunch, inoculated with oyster mushrooms, anoki or aspergillus. Samples results from the batch tests are shown in Figure 25.

Oyster Mushrooms demonstrated very strong growth on the sterilized paunch, with moderate growth also observed by aspergillus and enoki. The results demonstrate that fungi are able to utilize paunch as a substrate for growth. However, separation of the mushroom product and residual paunch was challenging. Therefore, reliable mushroom yields and biomass compositions were not determined and remain and area for further investigation.

Figure 25 Solid state fermentation on raw solid paunch conducted using batch laboratory experiments inoculated with oyster mushrooms (top), anoki (middle and aspergillus (bottom).

5.3.2 Raw Paunch Without Pre-treatment

The requirement for autoclave sterlisation has the potential to be expensive, energy intensive and involve a number of materials handling challenges. Therefore, the batch experiments were repeated at laboratory scale, using dewatered paunch with no pre-treatment. Samples results from the batch tests are shown in Figure 26. Batch tests using raw paunch were not successful, with very poor mushroom growth on raw paunch observed in all tests. Currently, it is not clear if the poor results on raw paunch are related to competition from native bacteria within the paunch or the presence of inhibitory compounds in the paunch liquor.

Figure 26 Solid state fermentation on raw solid paunch conducted using batch laboratory experiments inoculated with oyster mushrooms (top), anoki (middle and aspergillus (bottom).

5.4 Summary of Solid Waste to Protein Progress

Mushroom fermentation is an emerging technology that has been investigated for application to municipal solid waste. Potential advantages of mushroom fermentation include:

- Production of a relatively cheap source of high quality food protein using degradable cellulosic wastes
- Production of nutritionally enhanced dietary supplements/mushroom nutraceuticals (Mushroom Biotechnology),
- Bioconversion of difficult lignocellulosic materials into highly degradable bioenergy feedstocks
- Bioconversion/bioremediation of environmental contaminants (i.e. heavy metals).

Batch tests have been used to screen paunch as a growth medium for mushrooms, to date experiments have targeted oyster mushrooms, aspergillus and enoki mushrooms using raw paunch and paunch after autoclave sterilization pre-treatment. Initial results show very strong growth of oyster mushrooms on the sterilized paunch, with moderate growth also observed by aspergillus and enoki. These results could not be replicated on raw paunch with little or no growth observed. The results demonstrate that fungi are able to utilize paunch as a substrate for growth, however the process may be inhibited by native bacteria within the paunch or there may be a requirement for a physical pre-treatment (i.e. steam explosion) to change the structure of the paunch fibres and increase the bioavailability of the material.

The requirement for sterilization pre-treatment in the initial tests is a very significant practical challenge to be considered when assessing viability of the technology. This may be addressed through trialing alternative mushroom species; however a solution has not been confirmed at this time.

6 SUMMARY OF WASTE-TO-PROTEIN TECHNOLOGY APPLICATION PATHWAYS

This section describes the options for integrating waste-to-protein into Australian RMP, into the positioning of the SCP technologies within the broader waste treatment train.

6.1 Wastewater-to-Protein using PPB technology

This section provides a summary of process integration options for PPB technology at RMP plants, based on the results from this project. At this stage, technology integration is focused on the positioning of the PPB step within the treatment/value-adding process and not the specific configuration of the PPB reactor. Technology integration scenario 1 is shown in Figure 27.

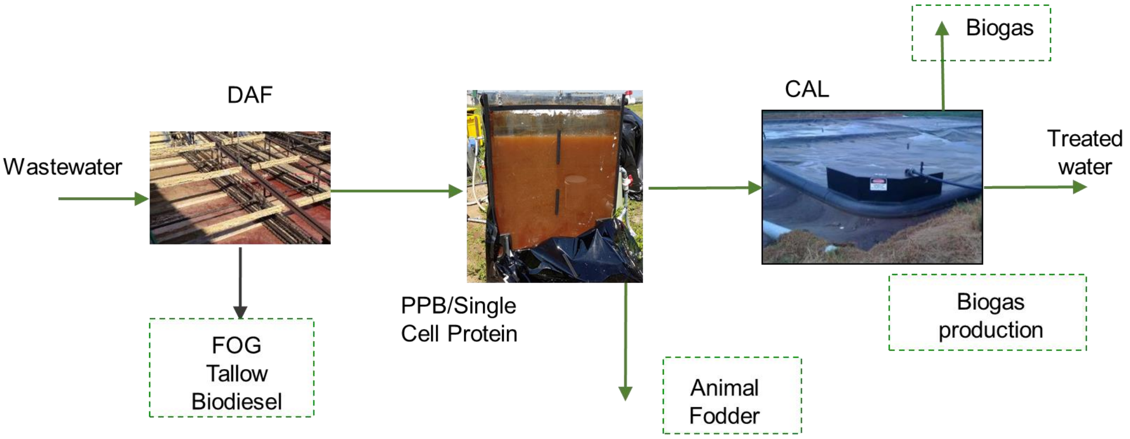


Figure 27: SCP Technology Integration 1. PPB Reactor placed after primary treatment. Excess carbon is converted to biogas using a CAL

In scenario 1, PPB technology is placed directly after existing primary treatment technologies, and treats wastewater with similar COD/N/P ratios and similar particulate/soluble fractions as the wastewater tested in this report. The major disadvantage of scenario 1 is that the wastewater is only partially available for PPB uptake and growth, results in this report demonstrate only 40-50% of nitrogen being converted to PPB, therefore only a portion of the value-add opportunity is achieved and additional treatment steps would be required to ensure the remaining COD and nitrogen were removed from the wastewater. The technology configuration is not efficient and may be strongly impacted by poor primary treatment, such as poor upstream FOG removal.

Technology integration scenario 2 is shown in Figure 28. In scenario 2, PPB technology is placed after RMP wastewater is first treated using a CAL. In this technology configuration, 80-90% of COD is removed from the wastewater and converted to biogas in the CAL. Importantly, nitrogen is not removed in the CAL and should pass to the PPB process as NH3, a form readily converted to protein by PPB. Over 90% of nitrogen removal is expected in this scenario if sufficient carbon is provided. In scenario 2, carbon is provided from 20% of the raw wastewater bypassing the CAL. The by-pass may require pre-fermentation due to the high fraction of particulate COD and poor availability of this material (demonstrated in this report). The pre-fermentation step was not assessed in the current project and requires development. A potential disadvantage of this Scenario is a 20% reduction in biogas energy from the CAL due to the bypass stream, however if the RMP was running a conventional biological nitrogen removal process, a bypass arrangement would be in effect to supply carbon for denitrification. A similar of lower bypass rate is required for PPB.

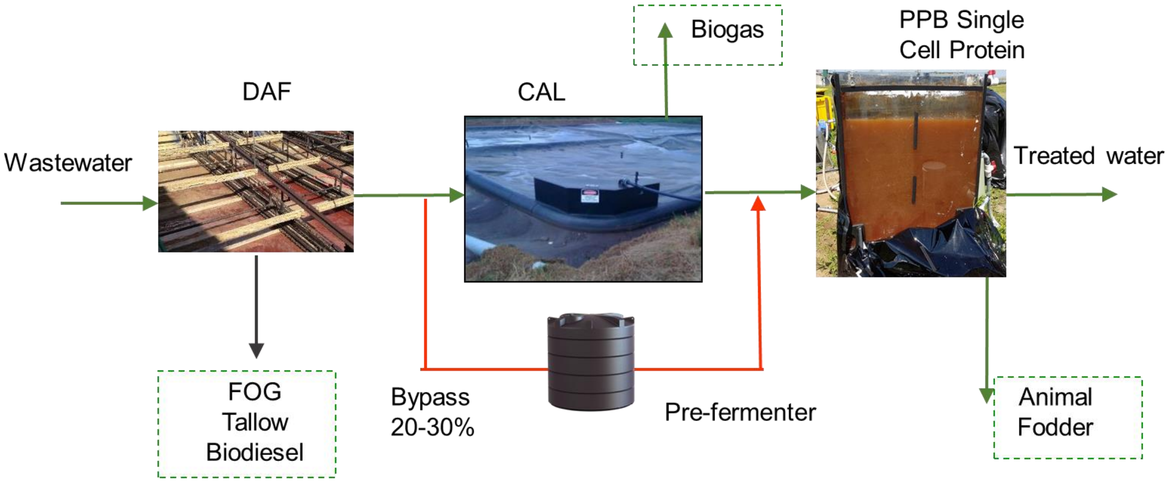


Figure 28: SCP Technology Integration 2. PPB Reactor placed after CAL. Carbon for PPB growth is supplied using a wastewater bypass configuration. Approximately 25% of wastewater would bypass the CAL.

Technology integration Scenario 3 is shown in Figure 29. Scenario 3 is similar to Scenario 2 in that the PPB technology is placed directly after a CAL and receives wastewater where all nitrogen has been mobilized as NH3. Again, over 90% of nitrogen removal is expected in this scenario if sufficient carbon is provided. However, in Scenario 3, carbon for PPB growth is sourced from DAF sludge separated during primary treatment. The added advantage of Scenario 3 is that this method does not impact on current biogas production levels and reduces a problematic solid waste in the form of DAF sludge. However, for Scenario 3 to be successful, the DAF sludge must be pre-treated to convert the FOGs into soluble organic acids. This pre-treatment step requires development.

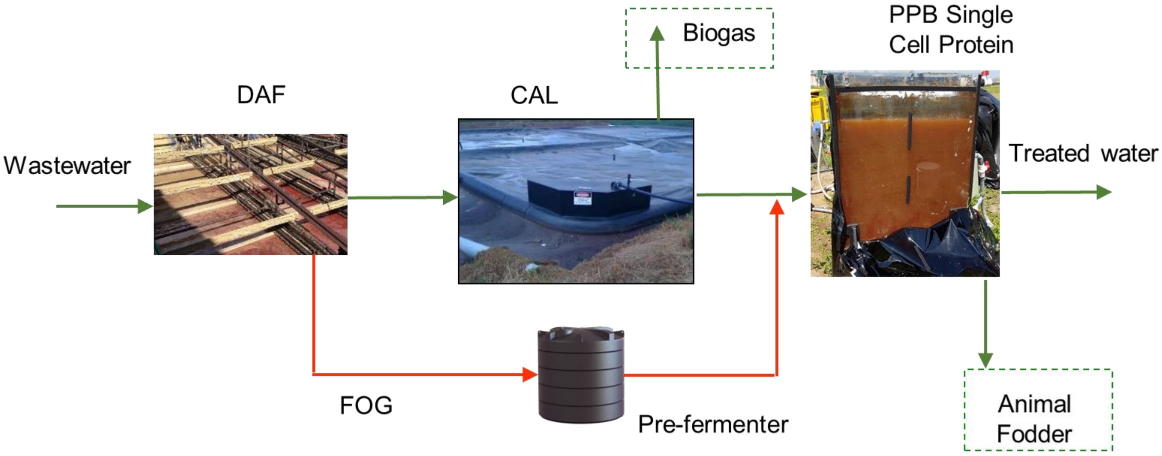


Figure 29: SCP Technology Integration 3. PPB Reactor placed after CAL. Carbon is supplied using DAF sludge after pre-fermentation.

6.2 RMP Solid waste-to-Protein using Fungal Fermentation technology

The solid structure of paunch solid waste is not readily available for protein production using PPB. Further, paunch solid waste contains relatively low nitrogen with a COD/N/P ratio of 100:1:0.3 being much lower than the 100:10:2 desired for PPB. The low nutrient content of paunch suggests a pre-fermentation step to solubilize the waste would not significantly enhance the feasibility of PPB. However, alternative solid-state fermentation technologies, such as mushroom fermentation demonstrate more potential for application to RMP solid wastes. Scenario 4, presented in Figure 30, demonstrates a technology application pathway for converting RMP solid waste into SCP. In Scenario 4, paunch solid waste is separated using existing dewatering equipment (rotating screens, fan press, screw press etc.), the dewatered solid waste is then sterilized prior to solid-state mushroom fermentation. The press liquor from dewatering can be passed to an anaerobic lagoon for energy recovery. While mushroom fermentation has been shown to occur on sterilized RMP solid waste, all steps in the flowsheet below require development before a reliable feasibility assessment can be completed.

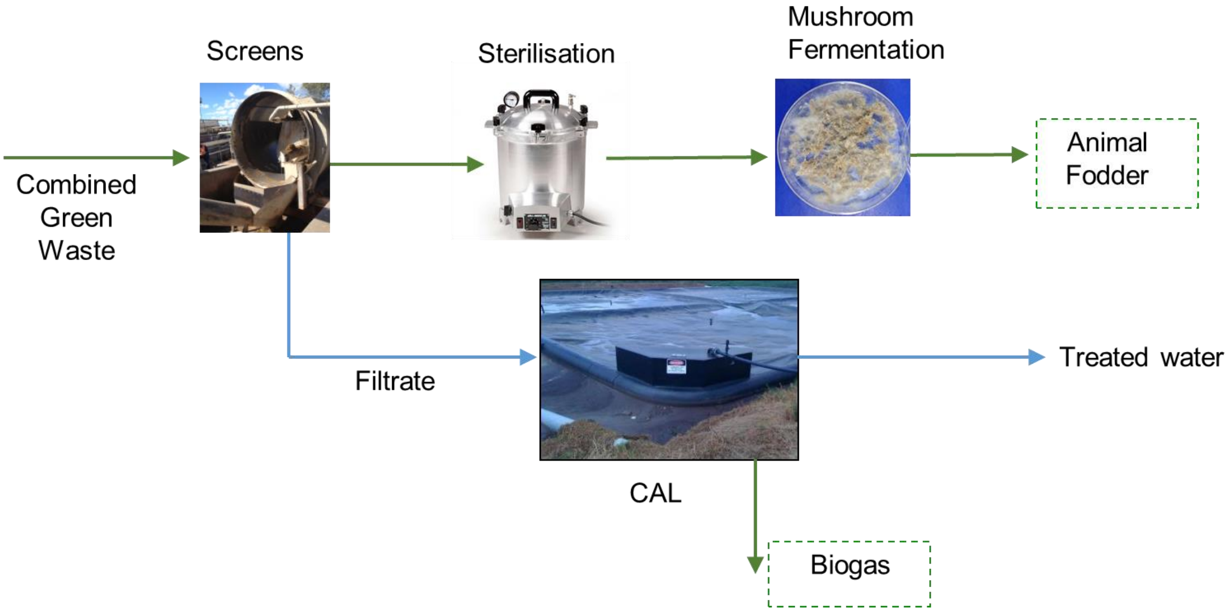


Figure 30: SCP Technology Integration 4. Solid-state fermentation of paunch solid waste after dewatering during primary treatment and pre-sterilization.

7 WASTE-TO-PROTEIN FEASABILITY

This section describes the current feasibility status for SCP technologies in the Australian red meat industry. The feasibility assessment includes development of PPB markets and product value; PPB production costs for the scenarios described in Section 6; comparisons to existing and conventional waste treatment technologies; and SWOT analysis.

7.1 Development of PPB Value

In this project, PPB biomass produced on RMP wastewater contained in the range of 65% crude protein for attached growth processes and 30-70% crude protein for suspended growth processes. These results are largely consistent with the composition of common species of PPB reported in literature and summarized in Table 4. The very high protein content makes PPB a key candidate for organic fertiliser applications (Xu 2001) and animal feed applications, particularly fish (Kobayashi and Tchan 1973) and poultry Ponsano et al. (2004). In addition to high protein content, PPB biomass contains a variety of useful products such as, vitamins, carotenoids, and ubiquinone (Takeno et al. 1999). While these compounds are potentially very high value, additional extraction and purification steps are required. Therefore, the value of PPB will be based on fertilizer and feed applications that utilise the bulk product.

Table 14: General composition of several PPB species.

	R. capsulatus[1]		**Rps. Gelatinosa**[2]		**R. gelatinosus**[3]	
	% 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
Crude fat	9.9	3.7	n.d	3.7	0.5	0.2
Soluble carbohydrates	20.8	3.5	n.d	3.5	25.6	4.3
Crude fiber	2.9	-	n.d	-	n.d	-
Ash	5.3	-	n.d	-	4	-
Total	-	17.4	-	18.1	-	15

Adapted from [1] (Blankenship et al. 1995a), [2] (Shipman et al. 1975), [3] (Ponsano et al. 2004), [4] (Adedokun and Adeola 2005), n.d = not determined.

The value of animal and aquaculture feed is generally related to feed-conversation ratio, animal mortality, and the nutrition value/quality of the end product. These parameters can require extensive feed trials to establish, therefore initial assessments of feed value are based on the dry matter ($ kgDM^{-1}), energetic value ($ MJ^{-1}) and crude protein (CP) costs ($ kg CP^{-1}). Based on Table 14, PPB has an average protein content of 60-65% and an average energetic value of 15-18 MJ kg^{-1}. Table 15 shows prices for common feed additives and standalone fodder materials. Based on this table, average costs were calculated, resulting in; $0.33 kg DM^{-1}, $0.02 MJ^{-1} and $1.7 kg CP^{-1}. MBM is included in the table for comparison, but is not included in the calculation. The value of Fish Meal is highlighted in the Table, due to previous research conducted as part of the Research Project: RnD4Profit Waste to Revenue: Novel Fertilisers and Feeds. APL (No. 2014/534.05). This project explored the use of PPB as a Fish Meal substitute in preliminary feed trials producing Barramundi. The trials showed no impact on fish mortality and limited impact on feed conversion ratio. These results, while preliminary, demonstrate the potential for PPB biomass to be used as a feed additive in aquaculture.

Table 15 includes a comparison of PPB and the corresponding energy/protein costs based on different values assigned to PPB biomass. In this comparison the value of PPB is presented over the range of \$0.1 kg CP^{-1} (representing a low-cost fertiliser scenario) to \$2.0 kg CP^{-1} (representing a Fish Meal substitute scenario).

Table 15: 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)**	**$\$\ t^{-1}$**	**$\$\text{cent kg DM}^{-1}$**	**$\$\text{cent MJ}^{-1}$**	**$\$\ \text{kg CP}^{-1}$**
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
Feed 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
Fish Meal 65% CP***	**87**	**13**	**68**	**2,000**	**200**	**15.4**	**2.9**
Source	**DM (%)**	**Metabolisable energy (MJ kg DM^{-1})**	**CP (% DM)**	**$\$\ t^{-1}$**	**$\$\text{cent kg DM}^{-1}$**	**$\$\text{cent MJ}^{-1}$**	**$\$\ \text{kg CP}^{-1}$**
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**	**1200**	**120.0**	**7.2**	**2.0**

*Source http://agriculture.vic.gov.au/agriculture/dairy/feeding-and-nutrition/cost-ofsupplements
**Source http://www.dpi.nsw.gov.au/agriculture/livestock/nutrition/values/price

*** Source https://www.indexmundi.com/commodities/?commodity=fish-meal&months=120

These values shown in Table 15 correspond to \$62 per dry ton and \$1200 per dry tonne respectively. Figure 38 demonstrates the value of 1.0 ML of red meat processing wastewater as water, energy (methane), nitrogen and phosphorous (top), compared to value of 1.0ML of RMP wastewater converted to PPB biomass. In this comparison, the energy value is largely preserved due to the high COD/N/P ratio in RMP wastewater and the excess carbon available. The composition of wastewater used in this analysis was 10,000 mg/L COD, 250 mg/L N and 50 mg/L P.

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 (Verstraete et al. 2009). When PPB is valued at $62 per tonne, the combined value of energy and PPB ($910) is actually lower than the combined value of energy and nutrients ($1060), largely due to the reduction in energy recovered from diverting carbon for PPB growth. However, if values of $600 per tonne ($2,200) or $1200 per tonne ($3,650) can be achieved for PPB biomass, the value of 1 ML of RMP wastewater rises substantially. Such values could be achieved if PPB is considered to have an equivalent value to MBM or a higher value as a fish meal substitute.

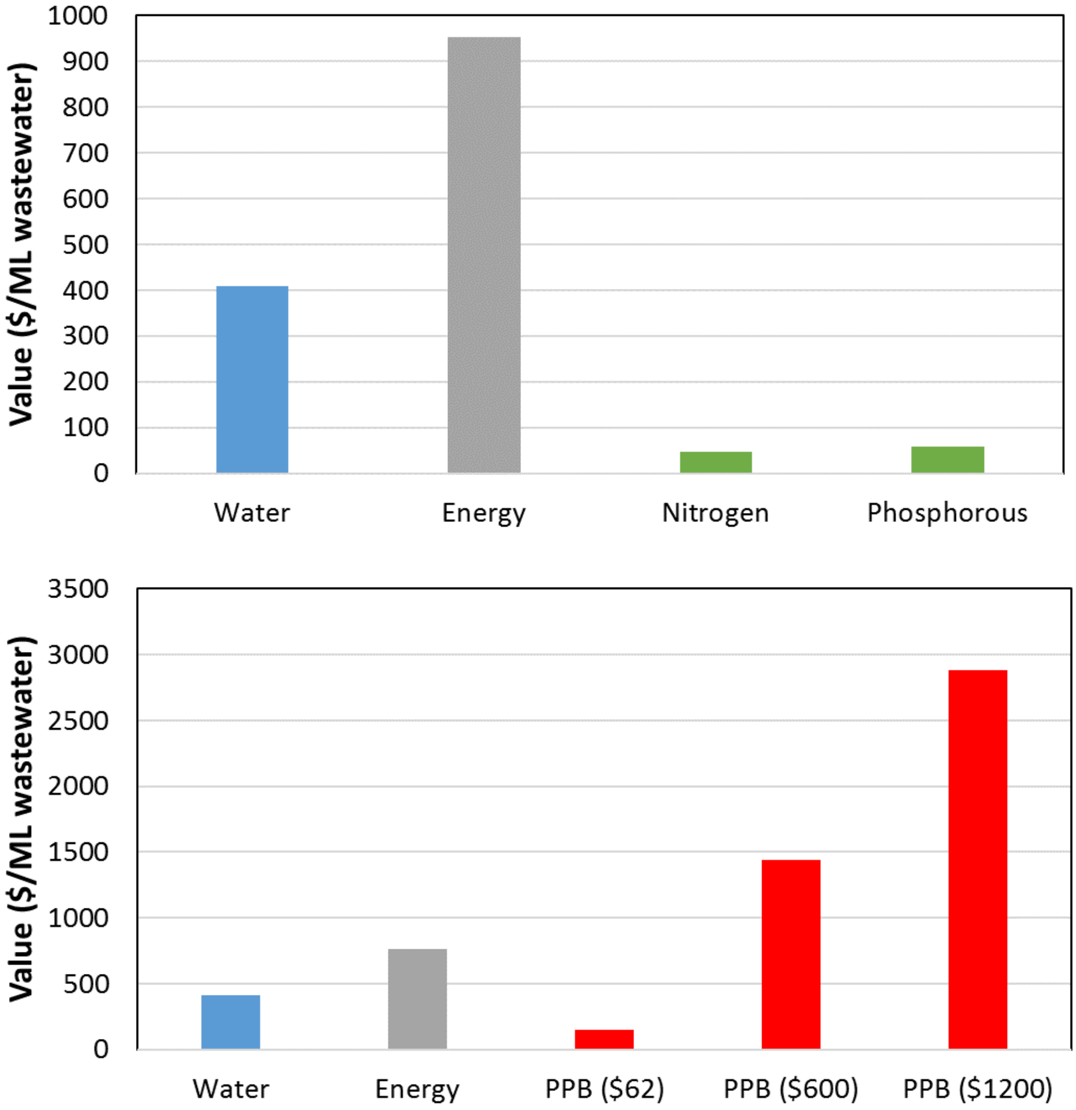


Figure 31: Potential value of 1.0 ML of red meat processing wastewater when recovering all resources as water, energy N and P (top); or as PPB biomass (bottom). The composition of wastewater used in this analysis is 10,000 mg/L COD, 250 mg/L N and 50 mg/L. Assigned values were: water ($0.41 m^{-3}), N ($0.19 kg^{-1}), P ($1.17 kg^{-1}) and methane ($10 GJ^{-1})(Verstraete et al. 2009).

7.2 Assessment of Wastewater Scenarios Implementing PPB

This section includes a basic assessment of potential wastewater treatment configurations for the Australian red meat processing industry. These options include the application of PPB scenarios described in Section 6 compared to conventional treatment technologies. Scenarios include:

- Covered anaerobic lagoon + BNR
- Covered anaerobic lagoon + anammox
- PPB + CAL + BNR
- CAL + PPB using bypass
- CAL + PPB using DAF sludge

The costing information in this analysis is expanded from AMPC project 2016-1023, which was the first project in this research program. 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 operating costs.

Capital costs are not included in the assessment due to the large uncertainty in the final design of the PPB process and the corresponding costs. Similarly the physical sizing of PPB reactors and ancillary equipment are still under development. 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.

7.2.1 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 1200 head of cattle per day, with total effluent flow of 3.46 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 16: Wastewater flow, concentration and load for case study the different process alternatives

	Concentration		Load	
Production level			1200	head d^{-1}
Wastewater volume			3400	kL d^{-1}
COD	10,000	mg L^{-1}	34,600	kg d^{-1}
Solids	3,480	mg L^{-1}	20,000	kg d^{-1}
Nitrogen	250	mgN L^{-1}	864	kg d^{-1}
Phosphorous	50	mgP L^{-1}	173	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.

Calculations for the PPB process are based on a HRT of 2 days, similar to treatment times demonstrated in this project. A HRT of 2 days for RMP applications would result in an organic loading rate of 5 kgCOD m^{-3} d^{-1} which is within the rates achieved for treatment of domestic wastewater using PPB. The illumination energy demand is estimated at 1 kWh.m^{-3}.d-^{1} The mixing energy is based on 0.15 kWh m^{-3} (Tchobanoglous et al. 2003). The harvesting cost for PPB biomass is estimated at \$0.5 per kg DS based on reported harvesting costs for Algae biomass (Fasaei et al. 2018).

Calculations for Covered Anaerobic Lagoons (CAL) were based on a treatment time of 20 days. During CAL treatment, 80% of COD entering the process was converted to biogas at a yield of 380 L CH_4 per kg converted. Energy recovery from the methane was 34 MJ/m^3 and was valued at \$10/GJ. Nutrients (N and P) were not removed in the CAL and were passed to the next treatment step.

Calculations for Biological Nutrient Removal (BNR) were based on a HRT of 2 days and a sludge age of 15-20 days. The carbon source for BNR was supplied by assuming 20% of wastewater bypassed the CAL. Energy demand was calculated as 4.6 kWh per kgN (removed as N_2) and 1 kWh kgCOD^{-1} that was oxidised Sludge production was estimated using a sludge yield of 0.3 on a COD basis. Sludge was dewatered to a cake solids content of 20% for disposal at an estimated cost of \$50 per wet tonne.

7.2.2 Treatment using Conventional Treatment Technologies

7.2.2.1 Covered Anaerobic Lagoon with Nitrification/Denitrification

Treatment using an anaerobic lagoon (CAL) followed by aerated lagoons or SBRs for nitrification and denitrification (BNR) is a wastewater treatment process commonly applied at many large RMP. Therefore, treatment using a CAL and BNR will represent the default treatment option (Figure 32). 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 off-site. A summary of operating costs for this default scenario (CAL + BNR) is shown in Table 17. The biogas revenue for the default process is $658,000. Aeration costs are $63,000 and sludge dewatering and disposal is $233,000.

Table 17: Example operating costs for a CAL followed by conventional BNR (Baseline Scenario 1)

	Basis	**Estimated Expenditure**
Operator support	0.5 FTE at $80,000	- $40,000
Covered Anaerobic Lagoon (55,000 kL reactor volume)		
Biogas Production	$10/GJ	$658,022
Biological Nutrient Removal (13,824 kL reactor volume)		
Aeration Energy	4.6 kWh per kg N and $0.1 per kWh	-$63,043
Sludge dewatering and disposal	$50 per wet ton (20% TS)	- $233,280
Total estimated operating		**$361,699**

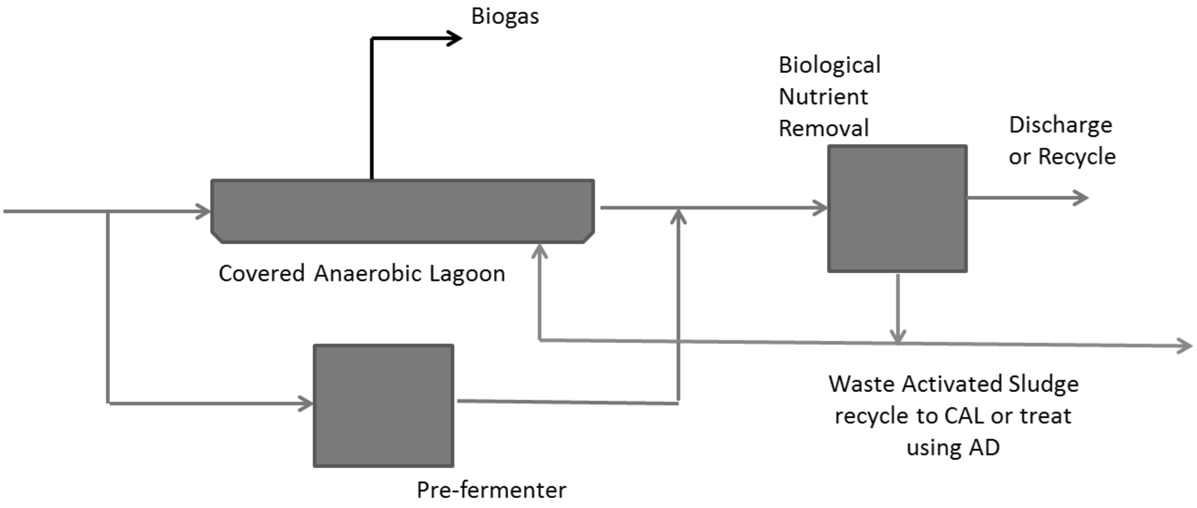


Figure 32: Process flowsheet representing a covered anaerobic lagoon followed by nitrification/denitrification in an SBR. The process is similar to that presented in ENV.044.

7.2.2.2 Covered Anaerobic Lagoon Coupled to Anaerobic Ammonium Removal

Anaerobic Ammonia Removal (AAR) 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 33. Design and costing of the covered anaerobic lagoon is similar to Section 7.2.2.1, however in this scenario wastewater does not bypass the CAL to provide carbon for nitrogen removal, this results in maximum biogas revenue of $822,000. The energy demand for N removal using the AAR process is significantly reduced at 1.2 kWh kgN^{-1} removed. In addition to N removal, the AAR reactor was assumed to oxidise a portion of the remaining COD. Energy demand for the COD removal was calculated at 1 kWh $kgCOD_{removed}^{-1}$. Sludge production in the AAR process is very low compared to BNR and sludge removal will happen infrequently. Sludge yields of 0.02 were assumed for calculations. A summary of operating costs for this low-cost nitrogen removal processes (CAL + AAR) is shown in Table 18. The biogas revenue for this scenario is $822,000. Aeration costs are reduced to $51,600 and sludge dewatering and disposal is comparatively small at $8,600.

Table 18: Operating costs for a CAL followed by low-cost N removal using AAR (Baseline Scenario 2)

	Basis	**Estimated Expenditure**
Operator support	0.5 FTE at $80,000	- $40,000
Covered Anaerobic Lagoon (55,000 kL reactor volume)		
Biogas Production	$10/GJ	$822,528
Biological Nutrient Removal (13,824 kL reactor volume)		
Aeration Energy	1.2 kWh per kg N and $0.1 per kWh	-$51,600
Sludge dewatering and disposal	$50 per wet ton (20% TS)	- $8,640
Total estimated operating		**$762,280**

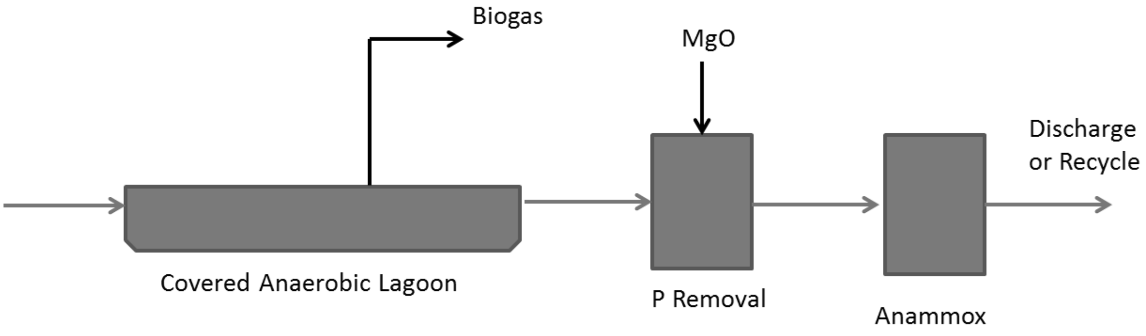


Figure 33: Process flow sheet representing covered anaerobic lagoon followed by anaerobic ammonium removal; Phosphorus removal is optional and is not included in cost calculations.

7.2.3 PPB technologies

7.2.3.1 Scenario 1 PPB followed by CAL and BNR

PPB Scenario 1 was previously shown in Figure 27. In scenario 1, PPB technology is placed directly after existing primary treatment technologies and before secondary treatment in a CAL, final nitrogen removal is achieved using an SBR. In this scenario, 40% of nitrogen is converted to PPB biomass at a crude protein content of 65%. A COD/VS ratio of the PPB biomass of 1.8 was used to calculate COD removal in the PPB step. After PPB treatment, 20% of wastewater bypassed the CAL to provide carbon for the final BNR process. During CAL treatment 80% of COD was converted to biogas energy, however N and P concentrations were not impacted.

A summary of calculated operating costs for PPB Scenario 1 are shown in Table 19. In this Scenario, biogas revenue is reduced to $584,000 as a portion of COD is consumed to produce PPB and a portion of COD is consumed during BNR. Final nitrogen discharge is lower in PPB Scenario 1 compared to the CAL+BNR baseline, however BNR costs are also lower. Lower BNR costs are because a portion of nitrogen is captured in the PPB reactor and converted to biomass product.

Table 19: Example operating costs for PPB placed after primary treatment (Scenario 1)

	Basis	**Estimated Expenditure**
Operator support	0.5 FTE at $80,000	- $40,000
PPB Process (6,900 kL reactor volume)		
PPB mixing Energy	0.1 kWh $m^{-3}d^{-1}$	- $25,920
Illumination energy demand	1 kWh $m^{-3}d^{-1}$	- $172,800
PPB Harvesting Costs	$0.50 per kg TS	- $392,727
PPB Product	3142 kg per day @ $0.1/kg	$78,545
	3142 kg per day @ $0.6/kg	$471,273
	3142 kg per day @ $1.2/kg	$942,545
Covered Anaerobic Lagoon (55,000 kL reactor volume)		
Biogas Production	$10/GJ	$584,742
Biological Nutrient Removal (13,824 kL reactor volume)		
Aeration Energy	4.6 kWh per kg N and $0.1 per kWh	- $66,053
Sludge dewatering and disposal	$50 per wet ton (20% TS)	- $173,428
Total estimated operating	**PPB Value $0.1 per kg**	- $167,640
Total estimated operating	**PPB Value $0.6 per kg**	$225,087
Total estimated operating	**PPB Value $1.2 per kg**	$696,360

Note: Operating costs included support personnel at 0.5 FTE for all scenarios ($80,000 per full time equivalent). Vessel and equipment maintenance costs are typically in the range of 4% of capital costs and have not been included in this assessment.

In Scenario 1, the value proposition of PPB technology is highly dependent on PPB value. At a PPB value of $62 per dry tonne, the PPB value is much lower than cost of PPB production. At a PPB value of $600 per dry tonne, the PPB revenue is still lower than the cost of PPB production, however the overall cost is approximately neutral due to BNR savings. At a PPB value of $1,200 per tonne, PPB revenue significantly exceeds the cost of production, with an approximate gain of $350,000. The major cost of PPB production is the harvesting cost and this is directly proportional to the mass of

PPB production. In Scenario 1, the illumination and mixing costs are proportionally high compared to PPB Scenario 2 and 3, this is because mixing and illumination are fixed by reactor size. A PPB Scenarios requires the same reactor volume; however the reactor is less efficient in Scenario 1 due to poor nutrient and carbon availability.

7.2.3.2 Scenario 2 CAL followed by PPB, using Bypass Carbon

PPB Scenario 2 was previously shown in Figure 28. In scenario 2, PPB technology is placed after RMP wastewater is first treated using a CAL. In this technology configuration, 80-90% of COD is removed from the wastewater and converted to biogas in the CAL. Importantly, nitrogen is not removed in the CAL and should pass to the PPB process as NH3, a form readily converted to protein by PPB. In the PPB reactor, 80% of nitrogen is converted to PPB biomass at a crude protein content of 65%. A COD/VS ratio of the PPB biomass of 1.8 was used to calculate COD removal in the PPB step. In scenario 2, carbon is provided from 20% of the raw wastewater bypassing the CAL.

A summary of calculated operating costs for PPB Scenario 2 are shown in Table 20. In this Scenario, biogas revenue is $658,000, which is approximately $100,000 higher than PPB Scenario 1 and is similar to Baseline Scenario 1. BNR is not required in PPB Scenario 2 and this is a substantial saving compared to the Baseline (approx. $300,000).

Table 20: Example operating costs for PPB placed after primary CAL using 20% bypass (Scenario 2)

	Basis	**Estimated Expenditure**
Operator support	0.5 FTE at $80,000	- $40,000
Covered Anaerobic Lagoon (55,000 kL reactor volume)		
Biogas Production	$10/GJ	$658,022
PPB Process (6,900 kL reactor volume)		
PPB mixing Energy	0.1 kWh $m^{-3}d^{-1}$	- $25,920
Illumination energy demand	0.5 kWh $m^{-3}d^{-1}$	- $172,800
PPB Harvesting Costs	$0.50 per kg TS	- $785,455
PPB Product	3142 kg per day @ $0.1/kg	$157,091
	3142 kg per day @ $0.6/kg	$942,545
	3142 kg per day @ $1.2/kg	$1,885,091
Biological Nutrient Removal (13,824 kL reactor volume)		
Aeration Energy	4.6 kWh per kg N and $0.1 per kWh	N/A
Sludge dewatering and disposal	$50 per wet ton (20% TS)	N/A
Total estimated operating	**PPB Value $0.1 per kg**	- $169,061
Total estimated operating	**PPB Value $0.6 per kg**	$616,393
Total estimated operating	**PPB Value $1.2 per kg**	$1,558,938

Note: Operating costs included support personnel at 0.5 FTE for all scenarios ($80,000 per full time equivalent). Vessel and equipment maintenance costs are typically in the range of 4% of capital costs and have not been included in this assessment.

Scenario 2, follows the trend of PPB Scenario 1 where the value proposition of PPB technology is highly dependent on PPB value. At a PPB value of $62 per dry tonne, the PPB value is much lower than cost of PPB production. Again, at a PPB value of $600 per dry tonne, the PPB revenue is similar to the cost of PPB production, although the overall cost comparison is positive due to BNR savings. At a PPB value of $1,200 per tonne, PPB revenue significantly exceeds the cost of production, with an approximate gain exceeding $1M. The major cost of PPB production remains the harvesting cost and this is directly proportional to the mass of PPB production.

7.2.3.3 Scenario 3 CAL followed by PPB, using DAF Carbon

PPB Scenario 3 was previously shown in Figure 29. Scenario 3 is similar to Scenario 2 in that the PPB technology is placed directly after a CAL and receives wastewater where all nitrogen has been mobilized as NH_3. Again, high levels of nitrogen removal are expected in this scenario if sufficient carbon is provided. However, in Scenario 3, carbon for PPB growth is sourced from DAF sludge separated during primary treatment. The added advantage of Scenario 3 is that this method does not impact on current biogas production levels and reduces a problematic solid waste in the form of DAF sludge. However, for Scenario 3 to be successful, the DAF sludge must be pre-treated to convert the FOGs into soluble organic acids. This pre-treatment step requires development.

In this scenario, 80% of nitrogen is converted to PPB biomass at a crude protein content of 65%. A COD/VS ratio of the PPB biomass of 1.8 was used to calculate COD removal in the PPB step.A summary of calculated operating costs for PPB Scenario 1 are shown in Table 21. In Scenario 3, biogas revenue increases back to the maximum of $822,000 as no pass is required and carbon is supplied from DAF sludge. All PPB costs/revenue in Scenario 3, are the same as Scenario 2, however the overall position is $170,000 more positive for all PPB values due to the higher biogas revenue.

Table 21: Example operating costs for PPB placed after CAL using pre-treated DAF sludge (Scenario 3)

	Basis	**Estimated Expenditure**
Operator support	0.5 FTE at $80,000	- $40,000
Covered Anaerobic Lagoon (55,000 kL reactor volume)		
Biogas Production	$10/GJ	$822,528
PPB Process (6,900 kL reactor volume)		
PPB mixing Energy	0.1 kWh $m^{-3}d^{-1}$	- $25,920
Illumination energy demand	0.5 kWh $m^{-3}d^{-1}$	- $172,800
PPB Harvesting Costs	$0.50 per kg TS	- $785,455
PPB Product	3142 kg per day @ $0.1/kg	$157,091
	3142 kg per day @ $0.6/kg	$942,545
	3142 kg per day @ $1.2/kg	$1,885,091
Biological Nutrient Removal (13,824 kL reactor volume)		
Aeration Energy	4.6 kWh per kg N and $0.1 per kWh	N/A
Sludge dewatering and disposal	$50 per wet ton (20% TS)	N/A
Total estimated operating	**PPB Value $0.1 per kg**	- $4,555
Total estimated operating	**PPB Value $0.6 per kg**	$780,898
Total estimated operating	**PPB Value $1.2 per kg**	$1,723,444

Note: Operating costs included support personnel at 0.5 FTE for all scenarios ($80,000 per full time equivalent). Vessel and equipment maintenance costs are typically in the range of 4% of capital costs and have not been included in this assessment.

7.3 Comparison of Red Meat Wastewater Treatment Options

PPB technology is also able to integrate with existing treatment processes, such as covered anaerobic lagoons and biological nutrient removal processes. The PPB 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 PPB 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 PBB process, however the COD must be pre-fermented to be available for PPB uptake. Table 22 is a summary comparison of the different treatment scenarios evaluated in the project. The costing information is not intended as a detailed feasibility analysis; it is intended as a preliminary comparison of the novel PPB technologies against current and emerging technologies for red meat processing wastewater. Further, the comparison gives an indication of the relative contributions of PPB production costs against PPB value and the impact of PPB processes on biogas revenue and/or nitrogen removal costs.

For PPB to economically feasible the PPB biomass has to be marketed as a high value organic fertiliser and/or as protein-rich feed additive. Based on this report, PPB could have a similar or higher value 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. However, PPB technologies become most attractive when the PPB product is marketed as a fish meal substitute in aquaculture feeds. Current market prices for Fish meal exceed $2,000 AUD (May, 2018). The highest value assigned to PPB is $1,200 per dry tonne and corresponds to value recovery exceeding $1M per year where all nitrogen can be converted to PPB.

In general, PPB technologies will reduce biogas revenue as a portion of COD is redirected from biogas production is consumed during PPB growth. However, this also occurs in a conventional BNR process where COD is consumed during nitrogen removal. PPB processes will also reduce nitrogen removal costs related to BNR aeration and waste sludge disposal. The comparison also shows that PPB production costs are higher than current nitrogen removal costs, and that a high portion of PPB production costs relate to illumination and harvesting. Illumination calculations were based on 0.5 $kWh.m^{-3}.d^{-1}$, this value is typical of laboratory and pilot operations, but is very high for a full-scale installation. Values of 0.05-0.1 $kWh.m^{-3}.d^{-1}$ are more typical of a full-scale installation, but must be validated. For some versions of PPB technology, illumination costs could be eliminated using sunlight with a filter to select for IR light. The most significant cost of PPB production is attributed to PPB harvesting, this is a major area for development and optimization.

Table 22: Comparison of Wastewater Treatment Case Studies

	Conventional Treatment		PPB		
Parameter	**CAL + BNR**	**CAL + AAR**	**PPB + CAL + BNR**	**CAL + PPB + Bypass**	**CAL + PPB + DAF**
Effluent COD	720 mg.L^{-1}	1600 mg.L^{-1}	535 mg.L^{-1}	327 mg.L^{-1}	463 mg.L^{-1}
Effluent N	50 mg.L^{-1}	50 mg.L^{-1}	30 mg.L^{-1}	50 mg.L^{-1}	50 mg.L^{-1}
Effluent P	34.6 mg.L^{-1}	49 mg.L^{-1}	29 mg.L^{-1}	32 mg.L^{-1}	31 mg.L^{-1}
Biogas Revenue	$658,022	$822,528	$584,742	$658,022	$822,528
BNR Aeration	-$63,043	-$51,600	- $66,053	N/A	N/A
BNR Sludge Disposal	$233,280	- $8,640	- $173,428	N/A	N/A
PPB Production	N/A	N/A	-$198,720	-$198,720	-$198,720
PPB Harvesting	N/A	N/A	- $392,727	- $785,455	- $785,455
PPB Revenue	N/A	N/A	$78,545 - $942,545	$157,091 - $1,885,091	$157,091 - $1,885,091
Annual Summary	**$361,699**	**$762,280**	**-$167,640 to $696,360**	**-$169,061 to $1,558,938**	**-$4,555 to $1,723,444**

Note: Annual Summary is based on PPB values ranging from $62 per dry tonne to $1,200 per dry tonne.

7.4 Waste-to-Protein: Strength, Weakness, Opportunity, Threat Assessment

This section describes a strength, weakness, opportunity and threat assessment for application of PPB technologies within the broader waste treatment train at RMP facilities.

SWOT Analysis **PPB for red meat wastewater treatment**	
Strengths • Non-destructive treatment • Organics, Nitrogen and Phosphorous recovered in a value stream • Potential for new revenue through organic fertiliser or protein rich feed product • PPB can integrate into existing treatment processes in different ways • Flexible reactor designs based on either biofilm or suspended growth models • PPB is compatible with existing bioenergy technologies – there is limited impact on biogas production • Proof-of-concept demonstrated for multiple agri-industry waste streams. PPB biomass contained >60% crude protein, this is a more concentrated form of protein than many existing feed supplements • Preliminary feed trials show no impact on fish mortality when PPB is used as a Fish Meal substitute	**Weaknesses** • Wastewater may require pre-treatment or pre-conditioning to ensure complete uptake of nitrogen • Specific reactor design requires development and optimization • Response to FOG is unknown – this creates risk due to unreliable primary treatment at Australian RMP • Harvesting costs are high, putting significant pressure on PPB value to achieve feasibility • Illumination costs are high, putting significant pressure on PPB value to achieve feasibility • Application and value of PPB biomass is uncertain – ability to tune or manipulate amino acid profiles and/or nutritional value is not certain • New market channels may be required • Legislation around PPB biomass use is poorly developed
Opportunities • Shift waste treatment operations to a resource recovery and value adding paradigm • Reduce or eliminate current problematic waste streams such as waste activated sludge and DAF sludge • Increase wastewater value 3-4x, potentially >$3,500 per ML	**Threats** • If loading rates cannot be achieved – capital costs may become excessive • If areal productivities are not achieved – capital costs may become excessive • If wastewater pre-treatment is not successful and/or if PPB is poorly integrated into a process, PPB production will not be efficient. Additional treatment

- Patents and IP development - Development of cross industry linkages to improve sustainability - May fit within established marketing channels e.g. MBM - Development of Global markets related to novel and/or sustainable feed products - Potential to tune PPB nutrition profile for certain markets - Significant opportunities remain for technology development and optimization.	steps will be required and costs may become excessive - PPB biomass not suitable as organic fertilizer – no market channels and/or poor product values - PPB biomass not suitable as feed additive – no market channels and/or poor product values - Regulatory environment changes and PPB from waste is prohibited as a feed additive – this significantly reduces PPB value and technology feasibility.

8 CONCLUSIONS AND RECOMENDATIONS

8.1 Protein from Wastewater

Purple Phototrophic Bacteria (PPB) are an emerging technology that enables the treatment of wastewater streams while producing potentially valuable feed or feed additives. In this technology, the removal of organics, nitrogen and phosphorus from wastewater occurs anaerobically in the presence of infra-red (IR) irradiation. Nutrients and organics are assimilated and/or accumulated by the PPB, which convert soluble compounds into a harvestable biomass with high protein content.

Previous research (2016/1023) established the proof-of-concept by growing PPB on RMP wastewater in laboratory batch tests. Research during this stage focused on the development of reactor designs for a continuous laboratory process. After initial feasibility assessments, 3 reactor configurations were identified for further development:

a) Continuous Photo-membrane Bioreactor
b) Attached growth reactor with internal illumination via hollow tubes; and
c) Mixed chamber set-up with illumination from the outside.

Continuous Photo-membrane bioreactors were subsequently discarded due a combination of complex design and operation, without the benefit of high quality product or concentrated product. PPB production from RMP wastewater was successfully achieved using both attached growth modes (such as biofilms) and using suspended growth modes. Attached growth resulted in a relatively consistent PPB product with high protein content (approx. 65%). Suspended growth resulted in more variable PPB product quality between 30% and 70% crude protein, this is partly attributed to the capture of wastewater particles in the product, which essentially dilute the product quality. While PPB product quality using is attached growth is higher and more consistent when compared to suspended growth, the attached growth reactors are more complicated and manual biofilm harvesting may be labour intensive. At larger scale, the suspended growth process could be implemented as a constructed bioreactor (using similar design principles to the lab reactors) or as a lagoon/raceway type system where a light filtering cover is used to supply the IR spectrum of sunlight. In this regard, the multi-chamber process is flexible enough to be implemented as either a higher-cost lower-footprint bioreactor option or a low-cost high-footprint lagoon option. With either technology, capture of nitrogen in the PPB product and subsequent conversion to microbial protein was limited, largely attributed to the form of nitrogen entering the reactors. The research focus is now optimizing PPB yields, through the use of pre-fermentation and/or pre-conditioning of waste streams.

There are multiple options for implementing PPB technologies into the wastewater treatment process at RMP, such as application directly after primary treatment or application after secondary treatment using a CAL. In general, PPB technologies will reduce biogas revenue as a portion of COD is redirected from biogas production is consumed during PPB growth. However, this also occurs in a conventional BNR process where COD is consumed during nitrogen removal. There is substantial potential to further optimize PPB technologies by reducing PPB production costs, this work is expected to target illumination costs through the use of filtered sunlight and/or harvesting costs through the use of biofilm or granular technologies.

For PPB to economically feasible the PPB biomass has to be marketed as a high value organic fertiliser and/or as protein-rich feed additive. Based on this report, PPB could have a similar or higher value 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. However, PPB technologies become most attractive when the PPB product is marketed as a fish meal substitute in aquaculture feeds. Current market prices for Fish meal exceed $2,000 AUD (May, 2018). The highest value assigned to PPB is $1,200 per dry tonne and corresponds to value recovery exceeding $3,500 per ML of wastewater treated.

8.2 **Protein from Solid Waste**

Paunch solid waste is a problematic solid waste stream at many Australian RMP. Paunch solid waste was considered as a feed stream for PPB production, however the high COD:N:P ratio of the paunch solid waste indicates that paunch does not contain sufficient nutrients for a high yield waste-to-protein technology such as PPB. Additionally, the paunch solid waste contained >95% particulate organic material, and this material is unlikely to be metabolized by PPB in the particulate form. However, the high solids content and high carbon content of paunch solid waste may be suitable for alternative waste-to-value technologies such as mushroom fermentation. Mushroom fermentation is an emerging technology that has been investigated for application to municipal solid waste. Potential advantages of mushroom fermentation include:

- Production of a relatively cheap source of high quality food protein using degradable cellulosic wastes
- Production of nutritionally enhanced dietary supplements/mushroom nutraceuticals (Mushroom Biotechnology),
- Bioconversion of difficult lignocellulosic materials into highly degradable bioenergy feedstocks
- Bioconversion/bioremediation of environmental contaminants (i.e. heavy metals).

Batch tests have been used to screen paunch as a growth medium for mushrooms, to date experiments have targeted oyster mushrooms, aspergillus and enoki mushrooms using raw paunch and paunch after autoclave sterilization pre-treatment. Initial results show very strong growth of oyster mushrooms on the sterilized paunch, with moderate growth also observed by aspergillus and enoki. These results could not be replicated on raw paunch with little or no growth observed. The results demonstrate that fungi are able to utilize paunch as a substrate for growth, however the process may be inhibited by native bacteria within the paunch or there may be a requirement for a physical pre-treatment (i.e. steam explosion) to change the structure of the paunch fibres and increase the bioavailability of the material.

The requirement for sterilization pre-treatment in the initial tests is a very significant practical challenge to be considered when assessing viability of the technology. This may be addressed through trialing alternative mushroom species; however a solution has not been confirmed at this time.

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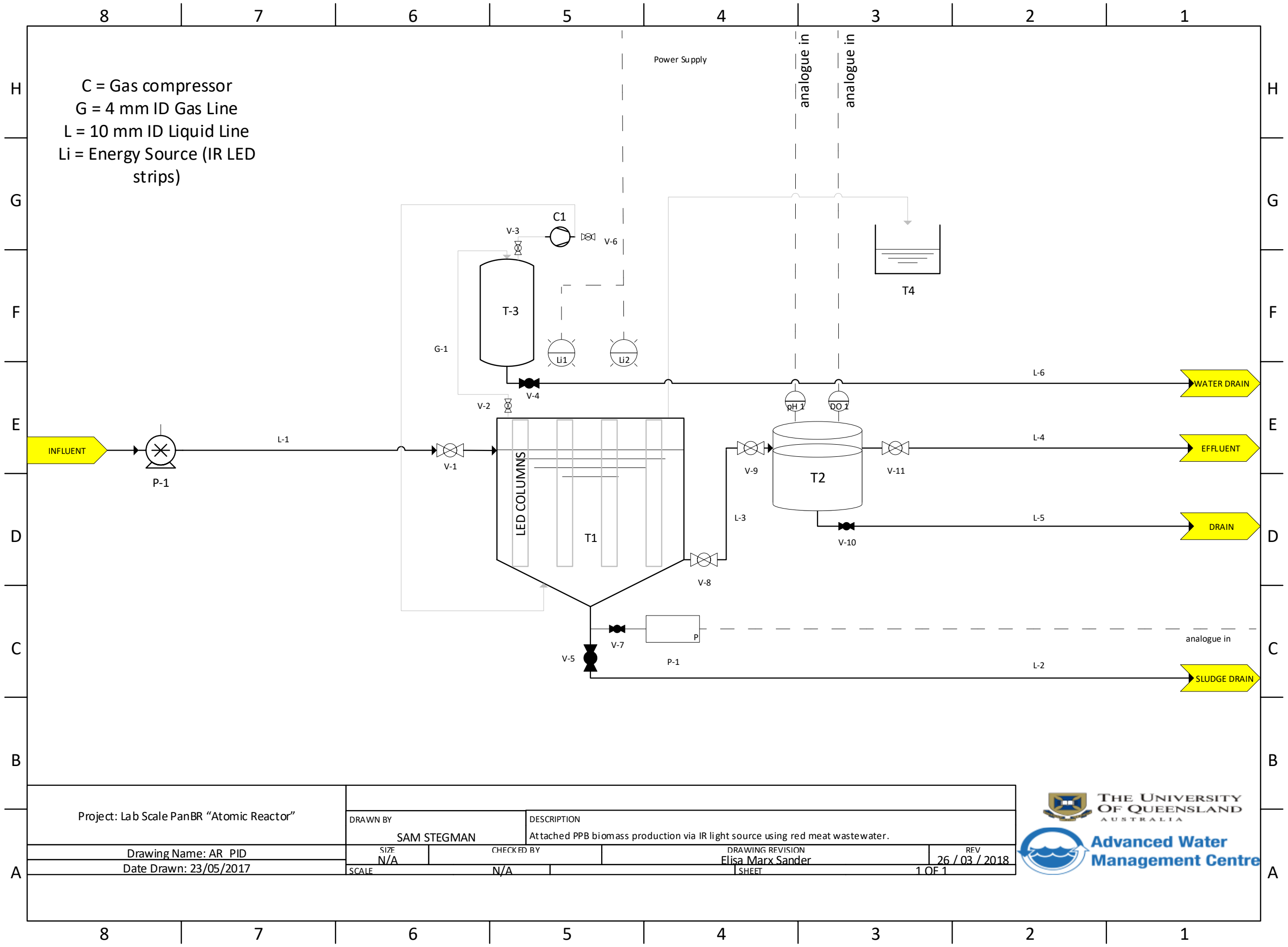
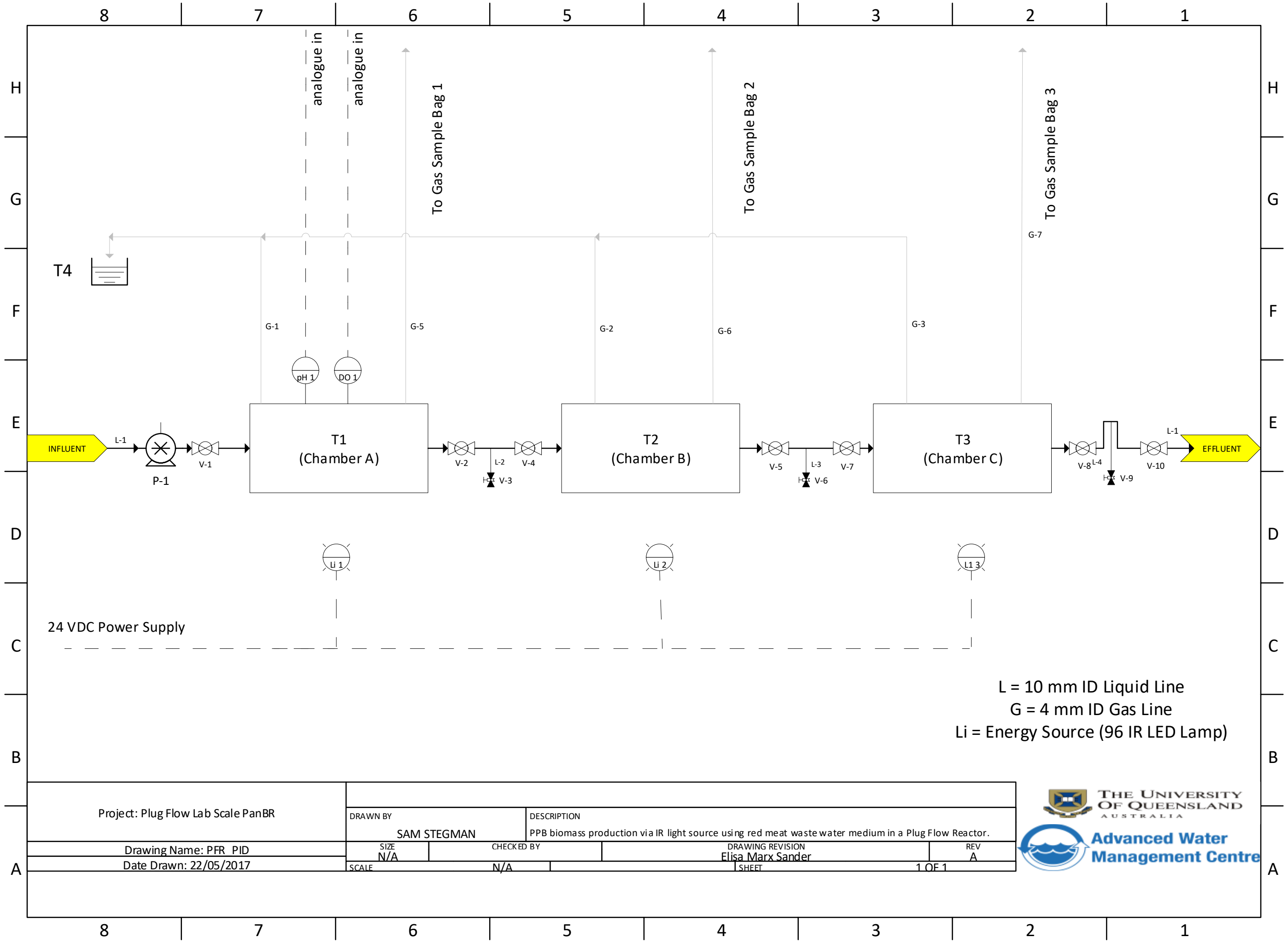
10 APPENDIX – ATTACHED GROWTH REACTOR



Project: Lab Scale PanBR "Atomic Reactor"
Drawing Name: AR_PID
Date Drawn: 23/05/2017
DRAWN BY SAM STEGMAN
DESCRIPTION Attached PPB biomass production via IR light source using red meat wastewater.
SIZE N/A
CHECKED BY
DRAWING REVISION Elisa Marx Sander
REV 26 / 03 / 2018
SCALE N/A
SHEET 1 OF 1

11 APPENDIX – MIXED LIQUOR REACTOR



L = 10 mm ID Liquid Line
G = 4 mm ID Gas Line
Li = Energy Source (96 IR LED Lamp)

Project: Plug Flow Lab Scale PanBR
Drawing Name: PFR_PID
Date Drawn: 22/05/2017
DRAWN BY SAM STEGMAN
DESCRIPTION PPB biomass production via IR light source using red meat waste water medium in a Plug Flow Reactor.
SIZE N/A
CHECKED BY
DRAWING REVISION Elisa Marx Sander
REV A
SCALE N/A
SHEET 1 OF 1

12 APPENDIX – PROGRESS LITERATURE AND TECHNOLOGY REVIEW

12.1 Summary

The main objectives of this review are listed below. The review will include coverage of current (conventional) technologies and approaches, national and international publications detailing the application of PPB and other phototrophic technologies and practices. The application of PPB will be highlighted and a cost-benefit analysis and comparison with current (conventional) practices as well as algal treatment systems will be carried out. The review includes a feasibility study to qualify the approach for applying PPB technology for wastewater treatment at red meat processing facilities.

The review includes (method):

1. Basic description of PPB treatment technology, with focus on current research and application with regards to identifying the potential for the application of PPB removal technologies for the red meat industry.
2. An explanation of how PPB technology might be applied to the meat industry – including the challenges and suitability of application
3. Comparison with current, conventional practices such as lagoons and anaerobic membrane bioreactor processes.
4. 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.
5. Market analysis to identify clearly value of potential products from red meat wastewater treatment with PPB.
6. An overview of the next likely R&D step(s) in relation to the above;

12.2 Background

Previous AMPC funded research has demonstrated that slaughterhouse wastewater has a relatively high organics to nitrogen ratio (A.ENV.0131, A.ENV.0151), and a very high biodegradability (A.ENV.0133, 2014/4007). The wastewater is potentially suited for an entirely novel process utilising infrared light to select for purple phototrophic bacteria (PPB). Under these conditions the PPB utilise organics, nitrogen, and phosphorous for microbial growth instead of metabolising these compounds for energy. In this process, energy for growth comes from light, instead of conversion of organics or nitrogen. This results in very high biomass yields, with essentially all the organics (carbon) and nitrogen partitioned into microbial biomass instead of being released as gas (carbon-dioxide, methane, nitrogen etc). Analysis of PPB grown from domestic wastewater has found the composition of the biomass product contains in the order of 65% protein content, 25% carbohydrates, remainder ash and lipids). The PPB process has no gas residue, and does not require energy intensive aeration, this is a significant improvement over conventional activated sludge processes. The PPB can then be harvested as a valuable product and this is another key difference between PPB processes and conventional activated sludge processes, where activated sludge is a waste product requiring disposal.

Within the existing project reports and literature, the PPB technology is most similar to the A-stage/anammox project, which uses chemical energy, instead of light energy to grow heterotrophic bacteria (A.ENV.0150). High rate aerobic treatment would require lower energy, but would not remove nitrogen effectively. A major advantage of PPB is the ability to produce a homogeneous microbial product due to selection by infra-red. The Advanced Water Management Centre (AWMC) has been unable to find current research in Australia, and very limited research worldwide on PPB for wastewater treatment. A patent search has been conducted, as part of the current domestic work. This has identified no relevant patents. Some patented methods for light delivery are relevant, but would enable application of technology, rather than restricting ability to operate (i.e., there are a number of methods for light delivery).

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12.4 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 contaminant also make red meat processing facilities are 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 Anammox allow economic removal of N, and are nearer to market, but do not offer the possibility for nitrogen or alternative 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 introduced by AMWC is a possible alternative, able to remove COD, N and P in one step.

12.5 Summary of Waste Production at Red Meat Processing facilities

Australian red meat processing facilities have potential to generate large volumes of wastewater and solid waste rich in organic contaminants and nutrients (Johns 1995, Liu and Haynes 2011). While potentially expensive, the removal of these contaminants is necessary in order to comply with water discharge regulations. Therefore red meat processing facilities are strong candidates for advanced treatment processes aimed at removal and/or subsequent recovery of energy, nutrient, and water resources.

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. A comparison of Table 1 and Table 2 shows that wastewater from Australian slaughterhouses is concentrated by international standards, both in regards to organic contaminants (COD) and nutrient (N and P).

Table 23 Composition of combined wastewater at Australian slaughterhouses compared with literature values

	Volume m^3 day^{-1}	TCOD mg L^{-1}	sCOD mg L^{-1}	TS^b mg L^{-1}	FOG mg L^{-1}	N mg L^{-1})	P mg L^{-1}
Literature Concentration^a	-	2,000-10,000	-	500-2,000	100-600	100-600	10-100
Site A	2420	12,893	1,724	8,396	2,332	245	53
Site B	3150	9,587	1,970	4,300	783	232	50
Site C	2110	10,800	890	7,530	3,350	260	30
Site D	2150	12,460	2,220	7,400	1,200	438	56
Site E	1600	10,925	1,195	6,118	1,569	272	47
Site F	167	7,170	1,257	3,806	1,915	182	27

c. Based on (Cowan et al. 1992, Johns 1995, Mittal 2004, Tritt and Schuchardt 1992)
d. Literature values are TSS (mg/L), study values are TS (mg/L)

Table 24: 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 mg L^{-1}	NH_4-N mg L^{-1}	TP mg L^{-1}
Borja et al. (Borja et al. 1994)	Spain	5,100	-	-	310	95	30
Caixeta et al. (Caixeta et al. 2002)	Brazil	2,000-6,200	-	40-600	-	20-30	15-40
Li et al. (Li et al. 1986)	China	628-1,437	-	97-452	44-126	25-105	10-16
Manjunath et al. (Manjunath et al. 2000)	India	1,100-7,250	-	125-400	90-150	-	8-15
Martinez et al. (Martinez et al. 1995)	Spain	6,700	2,400	1,200	268	-	17
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	30	15
Sachon (Sachon 1986)	France	5,133	-	897	248	-	22
Sayed et al. (Sayed et al. 1987)	Holland	1,500-2,200	-	-	120-180	-	12-20
Sayed et al. (Sayed and De Zeeuw 1988)	Holland	1,925-11,118	780-10,090	-	110-240	-	13-22
Stebor et al. (Stebor et al. 1990)	US	4,200-8,500	1,400	100-200	114-148	65-87	20-30
Thayalakumaran et al. (Thayalakumaran et al. 2003)	NZ	490-2,050	400-1,010	250-990	105-170	26-116	25-47

12.6 Wastewater Treatment

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). 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.

A general structure of wastewater handling practices is presented in Figure 1 and includes screening to reduce total suspended solids, dissolved air flotation (DAF) as a pre-treatment to remove fat, oil and grease (FOG) and further reduce 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 in tropical and equatorial temperate zones and engineered reactor systems (including activated sludge and UASB reactors) are commonly used in polar equatorial temperate zones. Anaerobic lagoons 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.

In the anaerobic step, proteins will be converted to biogas and the organic bound nitrogen will be realised 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, existing technologies can require energy intensive aeration steps and carbon chemical addition. Anaerobic ammonium removal technology is an emerging option to replace traditional nitrogen removal technologies at lower cost, however the focus of AAR is still removal. Single Cell Protein technologies, such as algae or PPB are alternative technologies designed to capture and transform nitrogen into a valuable product.

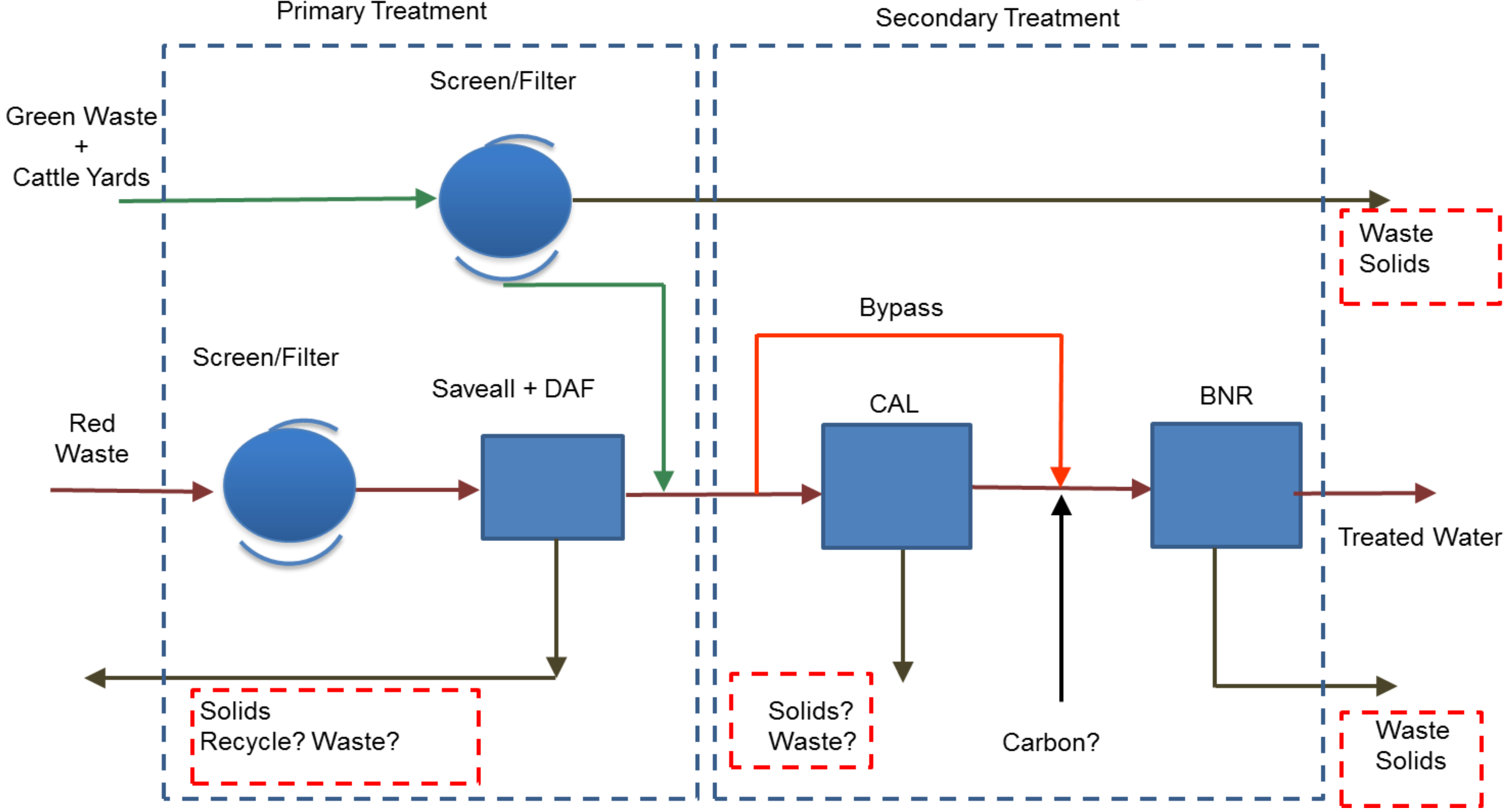

Figure 34: Example of waste handling process at Australian red meat processing facilities and major sources of organic solid waste (not including dead animals or packing wastes).

12.7 Solid Waste Collection and Handling

Solid waste originates from processing areas including paunch, manure, screenings (not rendered), DAF sludge, aerobic wastewater sludge, contaminated cardboard and condemned/dead animals. Cattle paunch in particular is a major waste produced at cattle slaughterhouses and is comprised of partially digested cattle feed, mainly containing grass and grain. The volume and composition of paunch waste varies according to individual animals and site handling practices but is reported at approximately 60 kg of wet paunch waste per animal (5-7 kg solids), corresponding to approximately 10% of the total weight of the live animal.

Current disposal methods for paunch and other solid wastes are largely based on composting, land disposal or landfilling. Direct land disposal is generally not considered a preferred option and is facing increasing regulation with application permits often required. Onsite composting is generally effective, but can require a large footprint and is a high-risk activity in terms of odour generation. Landfill can be a high-risk disposal method due to the landfill space availability and rapidly increasing landfill gate fees. Therefore, alternate disposal methods are required – with a preference for disposal methods that facilitate value recovery in the form of energy, nutrient fertilizers/organic mulches or other value add products.

AMPC has previously funded a series of research projects investigating the viability of using paunch waste as a boiler fuel (A.ENV.0110, A.ENV.120-123). Outcomes from these projects demonstrated some success when adding paunch to co-fuel the trial boiler, when the paunch was dewatered to TS content above 30% and mixed with a sawdust fuel at TS of 60%. The volumes of paunch combusted in these trials were much lower than the ratio of paunch produced at a typical meat processing plant, and the impact of paunch TS and paunch type (grass, grain etc.) were not reported. While there were some impacts on boiler efficiency, these short-term trials (approx. 3 hours) do demonstrate significant potential for paunch treatment through combustion-based technologies. However, there are a limited number of slaughterhouse's with the infrastructure to adopt the multi-fuel boiler approach.

Anaerobic digestion (AD) is an alternative approach to recover energy from paunch that was investigated through several AMPC/MLA projects (ENV.0068, A.ENV.0099, A.ENV.0155). The AD projects were successful at reducing the mass of paunch waste (60%) and recovering methane rich biogas (7 GJ/dry Tonne), however many conventional forms of AD were not considered economically attractive when applied to paunch. This was largely due to slow treatment times and high cost infrastructure requiring large capital investments. In both approaches, the economic success of the process appeared to be driven by a reduction in disposal costs, rather than the value of energy recovered.

Solid waste handling processes at RMP are generally include screening and dewatering technologies to reduce the volumes requiring disposal offsite. A brief summary of dewatering units and operational considerations are shown in Table 3. In plants using wet dump paunch handling processes, the solids content of paunch waste typically varies in the range of 5,000 mg/L TSS to 30,000 mg/L TSS prior to solids capture. The reported effectiveness of solids and nutrient capture during paunch dewatering processes varied between studies (MLA/AMPC 2012, 2013, MLA/AMPC. 2013). Generally 60-80% of paunch solids will be captured in the dewatered cake, however this may be increased to over 95% by adding chemical agents.

Recovery of phosphorus and potassium during dewatering is generally poor with 75-90% of P and K remaining in the wastewater filtrate. Recovery of nitrogen was more variable with 50-90% of N remaining in the wastewater filtrate, however nitrogen capture can be significantly improved by adding chemical agents during dewatering. Therefore, while paunch dewatering units as an effective strategy for reducing solids they are not an effective strategy for reducing nutrient loads.

Table 25: Summary of common equipment used for solids recovery from paunch

	Static Screen	**Rotary Screen**	**Screw Press**	**Degritting Hydrocyclone**
Capital Cost	Low ($15-20k)	Low ($15-20k)	Moderate ($50-80k)	Moderate ($50-80k)
Operating Cost	Low	Low	Moderate	Low
Life expectancy	Long	Long	Component replacement(s) after 10 years. Screens are subject to wear and may require replacement after 2-3 years	Moderate life
Application Area	Gross and Paunch Solids	All Solids	Paunch and Manure Solids	Stockyard Grit
Solids Cake	Wet	Wet	Dry (up to 50% solids)	Wet
Operating Weakness	Susceptible to hydraulic overloading and blockage	Susceptible to hydraulic overloading	Susceptible to damage from boluses or a lack of fibrous solids; damage from metallic objects in waste streams	Susceptible to blockage from paunch balls

12.8 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 contaminant also make red meat processing facilities are 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 Anammox allow economic removal of N, and are nearer to market, but do not offer the possibility for nitrogen or alternative 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 introduced by AMWC 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 35. 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.

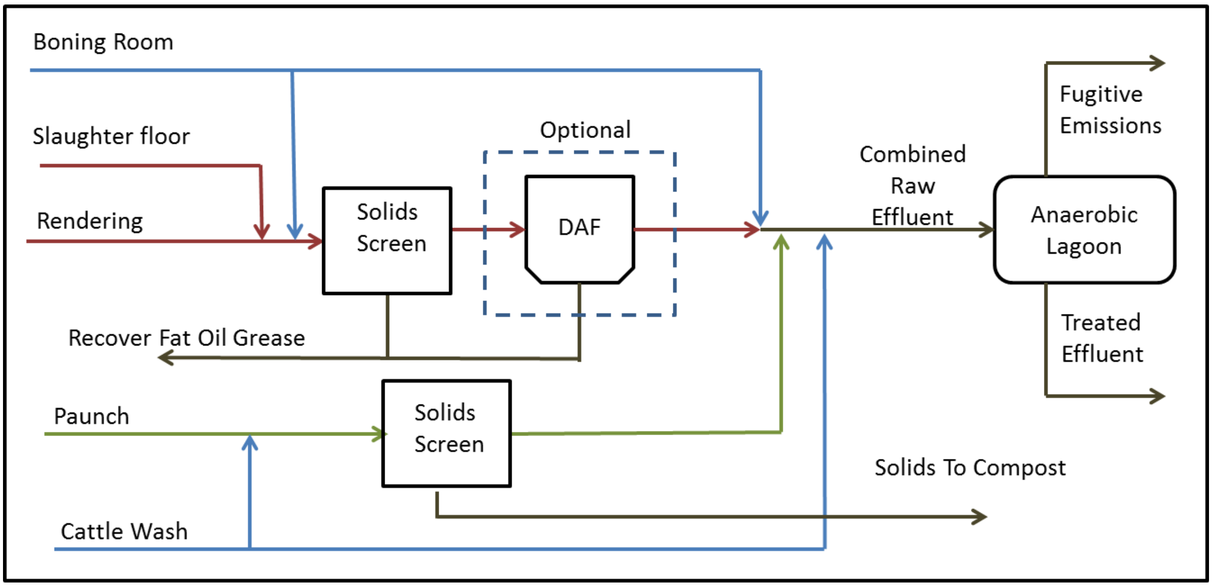

Figure 35: Major wastewater sources and generalised structure of waste and wastewater handling practices at Australian red meat processing sites (Jensen et al. 2014b)

The composition of combined wastewater at these Australian red meat processing facilities is shown in Table 26, while the compositions of slaughterhouse wastewater as reported in international studies are shown in Table 27. 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 26: 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 27: 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

12.9 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) (Figure 36).

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 in tropical and equatorial temperate zones and engineered reactor systems (including activated sludge and UASB reactors) are commonly used in polar equatorial temperate zones. Anaerobic lagoons 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.

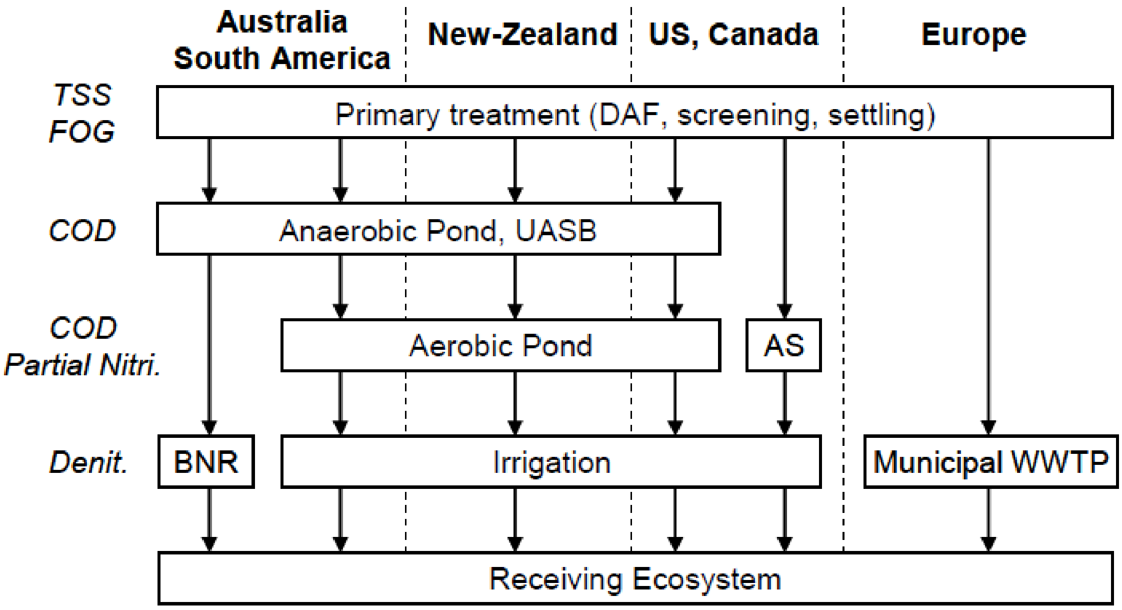

Figure 36: Principal wastewater treatment set-up of the meat industry (Lemaire 2007). Note: At some smaller Australian plants, primary treatment may be bypassed and/or raw effluent may be used for irrigation or land application.

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.

12.9.1 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 28.

Table 28: 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%

12.9.2 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 29.

Table 29: 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

12.10 **Current and Emerging Resource Recovery Technologies**

Common existing methods for managing RMP solid waste such as composting, direct land application and/or anaerobic digestion are either expensive, facing increasing environmental regulation or not applied to maximize the potential value in RMP waste, therefore effective and low cost management of RMP solid waste remains an industry wide challenge. Figure 37 is an example of current and emerging technologies to recover value from organic wastes, grouped into technologies for energy, nutrients and/or value-add products. The following section provides preliminary details on the technologies, including key products, technology readiness level (TRL – detailed in Appendix) and suitability for Red Meat Processing waste applications.

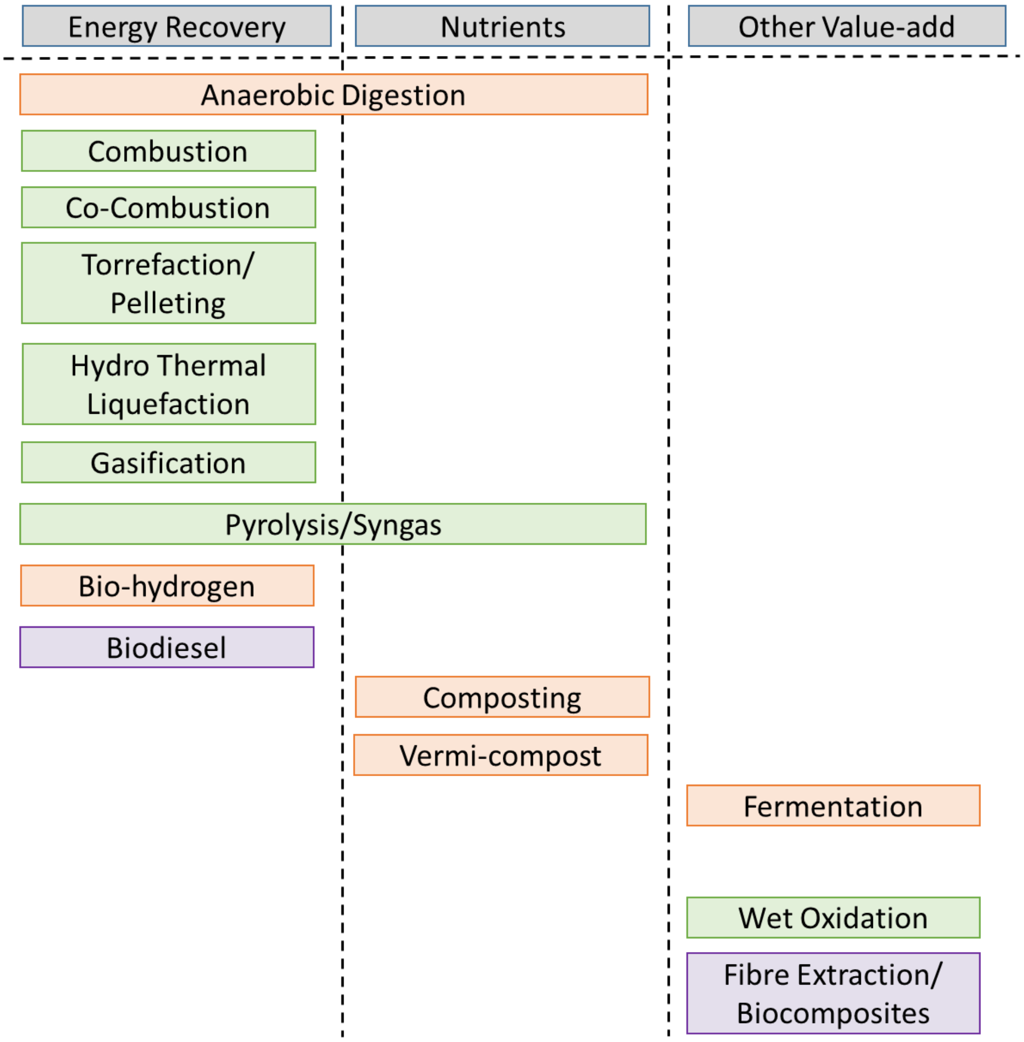

Figure 37: Summary of resource recovery technologies, grouped into energy, nutrient and/or alternative value-add applications (Orange – biological; Green – Thermal processing; Purple – other physical processes).

From a preliminary review of technologies, it is evident that treatment of solid waste is an area of active technology development with a clear shift in focus from waste disposal to resource recovery technologies. Although, there are many technologies under development, anaerobic digestion followed by land application is the most commonly considered for value recovery across the broadest range of industries in Australia and internationally, however this technology requires further optimization to improve the economics. Thermal processes, such as co-combustion are more readily applied internationally due to very strict regulations around landfilling and land application. Thermal processes are rarely applied in Australia, but new technologies with lower costs are emerging.

12.10.1 **Resource Recovery Technologies – Energy Recovery**

Table 30 provides a summary of energy recovery technologies, including key products, technology readiness level (TRL – detailed in Appendix) and suitability for red meat processing applications.

Table 30: Summary of Resource Recovery Technologies used for Energy Recovery

Name	Description	Products	TRL	Suitability RMP
Anaerobic digestion (Appels et al. 2008) For: All RMP organic wastes	Biological process that occurs in the absence of oxygen and converts organic material into biogas (methane-rich gas) and a stabilized digestate in the absence of oxygen. Generally less than 50% of solid waste will decompose and be converted to biogas Technically applicable to all RMP wastes, however the feasibility of this technology depends on the biological degradability of the material – determined using BMP testing. Methane yields and digestate stability are subject to sludge characteristics and process operating conditions. May be implemented as low cost lagoons, however addition of RMP solid waste to lagoons is not recommended as lagoons will accumulated solids rapidly and require expensive dredging, desluding and disposal operations. May be implemented as invessel AD, which is widely implemented at medium/large STPs. Conversion of solids is still approximately 50%, however the process is designed for constant removal and separate of the digestate. In vessel digestion is generally more expensive than lagoon technologies. AD residues may be applied to land as an organic fertiliser or further treated using thermal processing technologies.	Biogas (E) Biosolids (N)	9	High
(Co-) Combustion (Donatello and Cheeseman 2013, Fytili and Zabaniotou 2008, Husillos Rodríguez et al. 2013) For: All RMP organic wastes	Thermal process where the organic content of waste is oxidised into CO_2 and H_2O and heat energy is released. Requires excess oxygen concentration (related to COD). Can be applied to raw dewatered sludge or residues after AD. Moisture does not contribute chemical energy, but vaporises and consumes the heat released during combustion. Therefore, wastes generally needs to be dried for efficient combustion. Important consideration include: Adequacy of the combustion chamber to manage the volatility of the dried sludge. Adequacy of the gas treatment line to handle the higher NO_X and particles emissions. Mineral content of waste generally remains as ash. Ashes are potentially used on land as fertilizer or incorporated in cements, brick, etc.	Thermal and electrical energy Ashes (N, V_{add})	8	Medium

Name	Description	Products	TRL	Suitability RMP
	Combustion achieves the largest reduction of the SS volume. Most commonly applied outside Australia and at centralised facilities with low land availability.			
Torrefaction/ Pelleting (Li et al. 2015) For: All RMP organic wastes	Thermal process (200 – 400 °C) used as a pre-treatment or conditioning step prior to further thermal processing, such as combustion or pyrolysis. Torrefaction dries waste and removes volatile compounds with lower calorific value. The resulting pellets are generally dry and energy dense. Pellets are easier to transport and store. The waste pellets after torrefaction have better properties for energy generation than just dewatered and dried waste. Technology already used for other types of biomass. It can be co-pelletised with other biomass/waste. Existing market demand for pellets as multi-fuel boiler feed.	Pelleted Biomass/ fuel (V_{add})	6	Medium/ Low
HydroThermal Liquefaction For: All RMP organic wastes	waste decomposition at high temperature (300-400 °C) and pressure (~200 atm) into biocrude oil. Dewatering or pre-drying of waste is not required. Feedstock flexible. Bio-oil properties are comparable to fossil crude oil. Promising technology under development. But limited commercial application and this increases risk. Requires energy input. Biocrude yields and energy balances need to be assessed.	Bio-oil (E)	5	Medium/ High
Gasification (Fytili and Zabaniotou 2008) For: All RMP organic wastes	Thermal process that decomposes organic matter at elevated temperature (1000 °C) at a limited oxygen concentration. Gasification generally produces are larger volume of ash than oxygen rich combustion, and may produce a value-add char. A portion of syngas is often used to supply energy to the process. The energy balance is highly sensitive to moisture content and the waste needs to be dried before thermal processing. Technology under development. Few applications worldwide.	Syngas (CO & H_2) Ashes	6	Medium

Name	Description	Products	TRL	Suitability RMP
Pyrolysis/Syngas (Fonts et al. 2012) For: All RMP organic wastes	Thermal process that decomposes organic matter at elevated temperature (300-800 °C) in the absence of oxygen. Product proportions are affected by process conditions and feedstock composition. Biochar makes the heavy metals in the waste more resistant to lixiviation. The energy balance is highly sensitive to moisture content and the waste needs to be dried before thermal processing.	Syngas Tar/Oil (V_{add} / E) Char (solid) (V_{add} / E)	6	Medium/ High
Bio-hydrogen For: All RMP organic wastes	Biological process that degrades waste into a hydrogen-rich biogas. Needs to be followed by anaerobic digestion. Energy balances and cost benefit are not clear. Limited evidence that the additional capital and operation costs are offset by the energy production. Bio-hydrogen production can be coupled with fermentation.	Biogas (E) Carboxilic acids (V_{add})	6	Medium/ Low
Biodiesel For: DAF sludge and other high fat streams	Production of a biodiesel after extraction of fat, oil and grease from waste (note: this is a different form of oil to the bio-crude generated from some thermal processes). The low lipid yields in the waste combined with the high cost of the extraction process makes this technology unsuitable to most sludge streams. The technology is suited to wastes with high lipid content.	Biodiesel (E)	8	Low

Notes: E: energy recovery; N: nutrient recovery; V_{add}: value-add product; GHG: greenhouse gases.

12.10.2 Resource Recovery Technologies – Nutrient Recovery

Table 31 provides further details on nutrient recovery technologies, including key products, technology readiness level (TRL – detailed in Appendix) and suitability for sewage sludge applications. Technologies that enable energy recovery and nutrient recovery, such as anaerobic digestion and pyrolysis are included in Table 30 and not repeated in Table 31.

Table 31: Summary of Resource Recovery Technologies used for Nutrient Recovery

Name	Description	Products	TRL	RMP suitability
Composting (Wei et al. 2001)	Microbial process that converts waste into compost (stable organic matter). That are multiple technologies configurations, many are low-tech low-cost technologies. All forms of composting produce a stable final material that can be sold or applied as a soil conditioner. However, the value of the compost may be lower than the cost of production. Pre-drying waste or blending with dry materials may be required. GHG emissions and odours may be high. Energy content is not recovered, however nutrients may be recycled as compost product. Commercially applied in RMP (particularly Australia), but facing increasing regulation and diminishing practice in favour of anaerobic digestion and co-combustion.	Compost (N)	9	Medium/High
Vermi-compost	Vermicomposting is a variation on the composting process using various species of worms. The resulting vermicast is claimed to be of high value. However, the process requires significant investment and ongoing management. Specific performance will depend on the waste characteristics and the species of worms or larvae grown. Energy content is not recovered, however vermi-compost is generally rich in water soluble nutrients and is a good organic fertilizer/soil conditioner. Several operations have been successful whereas others have failed. There is ongoing interest in the use of black solider flies to produce protein from RMP wastes.	Compost (N)	8	Medium/High
Surface Spreading	Direct land application on the land surface. This method may be applied on the raw waste, or waste after stabilisation through composting or anaerobic digestion. Direct application of raw waste was widely used in the past due to low costs. However, this method is facing increasing regulations and permitting requirements.		9	
Sub-surface Injection	Direct soil injection may be applied on the raw waste, or waste after stabilisation through composting or anaerobic digestion. This method involves mixing the waste within the soil layer and still requires EPA approval. This method is more expensive than surface spreading, but has the added advantage of reducing the fly problem but the long term effect on the soil should be monitored.		9	

Notes: E: energy recovery; N: nutrient recovery; V_{add}: value-add product; GHG: greenhouse gases.

12.10.3 Resource Recovery – Alternative Value-add technologies

Table 32 provides further details on alternative value recovery technologies, including key products, technology readiness level (TRL – detailed in Appendix) and suitability for red meat processing applications. There are several emerging options for producing high protein feeds from waste, however much of the research is preliminary. The technology configuration and the technology readiness is not clear for RMP applications, similarly market development for the product requires significant development.

Table 32: Summary of Technologies used for Alternative Value-add Applications

Name	Description	Products	TRL	RMP suitability
Fermentation	Partial-degradation of the waste by microorganisms to produce carboxylic acids. Low-tech low-cost technology. However, fermented waste generally requires further treatment before disposal. Fermented waste is compatible with AD and some thermal processing technologies. The carboxylic acids produced can be used as a carbon source for biological nutrient removal, converted to energy in anaerobic digester, converted to bioplastics or possibly sold as commodity chemicals.	Carboxylic acids (V_{add})	5	Medium/Low
Wet Oxidation (Baroutian et al. 2015, Bertanza et al. 2015)	Decomposition of waste at moderate temperature (150-350 °C) and high pressure (20-150 atm) using pure oxygen or air. Currently used to treat industrial wastewater with recalcitrant compounds. Pre-drying waste is not required. The carboxylic acids produced can be used as a carbon source for biological nutrient removal, converted to energy in anaerobic digester, converted to bioplastics or possibly sold as commodity chemicals. Although there are approx. 250 reference plants worldwide, there are still considerable knowledge gaps for RMP applications.	Carboxylic acids (V_{add})	8-9	Medium
Bioplastics	Biological process that forces the accumulation of Polyhydroxyalkanoates (PHA) in bacterial cells. PHAs are used in the production of biodegradable bioplastics. Requires changing the STP operational conditions, including SS fermentation. Plastic yields are typically low and high recovery costs hinder process feasibility. After plastic recovery SS stills needs to be treated before disposal. Technology in its early stages.	Bioplastic (V_{add})	5	Medium/Low

Name	Description	Products	TRL	RMP suitability
Fibre Extraction/ Biocomposites	Sludge may be rich in undigested lignocellulose fibers. Such fibers could have value in making paper, board and biocomposite materials (applications including automotive, packaging, furnishings, decking, etc.). Fibres generally refined using chemical digestion (K/NaOH)	Biocomposites (V_{add}) Waste Application	9 2	Medium
	Many RMP include primary treatment to separate waste high in fat, oil and grease. In some cases, the wastes can be recycled to rendering to produce lower grade tallow. However, depending on the primary treatment method, the by-products may be excluded rendered products from certain markets.		9	High (to FOG streams only)

Notes: E: energy recovery; N: nutrient recovery; V_{add}: value-add product; GHG: greenhouse gases.

AMPC

12.11 Purple phototrophic bacteria for wastewater treatment

12.11.1 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 33 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. 1995b). 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. 1995b). Further readings about the biochemistry and molecular structures of the light harvesting complexes (LHC) can be found elsewhere (Madigan and Martinko 2006, McEwan 1994).

Table 33: 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.

12.11.2 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 34 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 34: Summary of wastewater treated with PPB.

Wastewater	Pre-treatment	COD$_{removed}$	PO$_4$-P $_{removed}$	NH$_4$-N$_{removed}$	HRT	Light	PPB	Ref
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. spaeroides	(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)

12.11.3 End use and value of PPB

PPB contain a variety of useful products such as, vitamins, carotenoids, ubiquinone (Takeno et al. 1999) and proteins (Chiemchaisri et al. 2007). Carotenoid pigments are another potential valuable bio-product. Carotenoids can be used commercially as vitamins, antioxidants and for cancer chemoprevention (Naves and Moreno 1998). 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 (2001) 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 (Kobayashi and Tchan 1973).

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 (Kobayashi and Tchan 1973). The same study reported increased egg production in hens with PPB biomass as feed additive. Ponsano et al. (2004) reported the use of PPB as poultry feed.

The composition of PPB is shown in Table 35 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 (Matassa et al. 2015). 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 (Matassa et al. 2015). 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 35. 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 35: 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] (Blankenship et al. 1995a), [2] (Shipman et al. 1975), [3] (Ponsano et al. 2004), [4] (Adedokun and Adeola 2005), 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: (Source)).

Table 36: 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
Feed 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:*(Source), **(Source)

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 38 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 (Verstraete et al. 2009). The value of the PPB biomass does not include the revenue for potential water recovery.

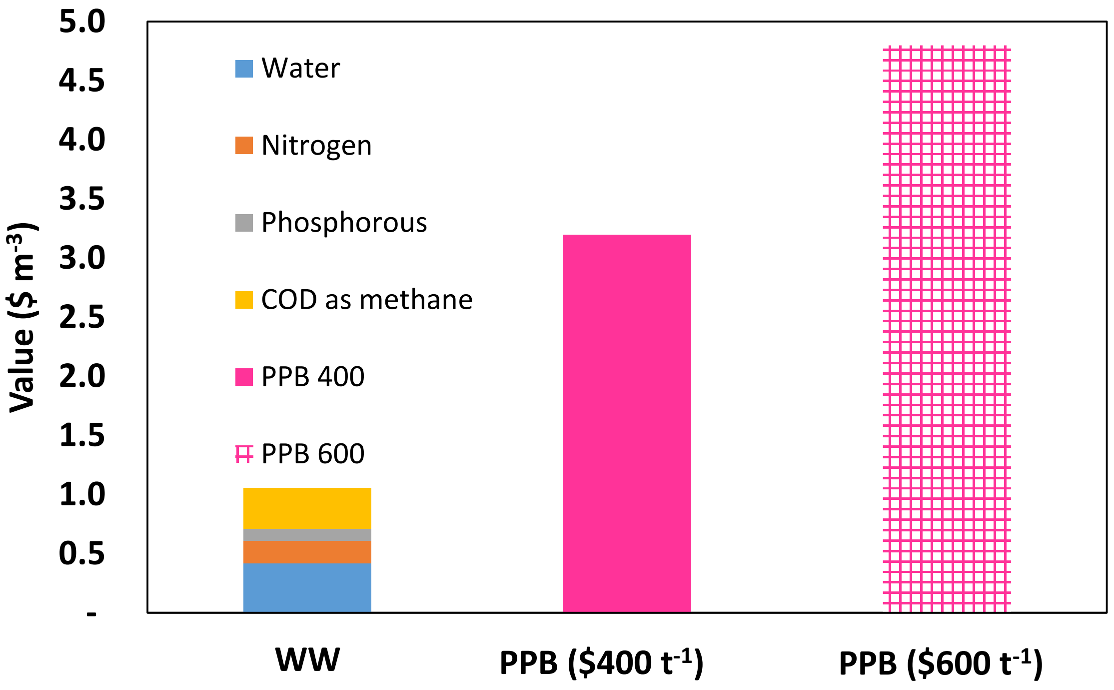

Figure 38: Potential value of 1.0 m^3 of red meat processing wastewater when recovering all resources (WW), selling the biomass at \$400 t^{-1} and \$600 t^{-1}.

12.12 Application of PPB for red meat 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.

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 (Kim et al. 2004). 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 (Azad et al. 2004). 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 26 and Table 27) 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 39 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.

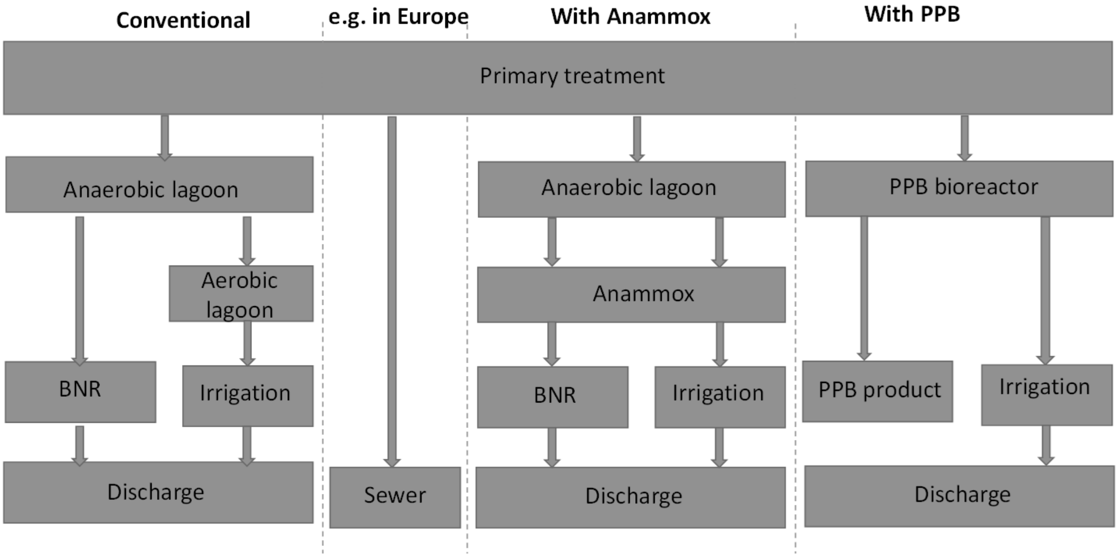

Figure 39: Comparison of treatment option for red meat wastewater and principal location of treatment steps.

12.13 Comparison to competing technologies focused on protein recovery from waste

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 (Posadas et al. 2015). However, more recent advances target application to primary wastewater with a focus on simultaneous C, N and P removal for recovery (Henkanatte-Gedera et al. 2015).

Waste stabilisation ponds (WSP) as described by Oswald et al. (1957) 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 37: 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)(Godos et al. 2009), 2) (National-Research-Council 2012), 3) (Shoener et al. 2014)

12.13.1 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 (Borowitzka 2013).

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 (Borowitzka 2013). 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* (Cysewski and Lorenz 2004). 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 (Pulz and Gross 2004).

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 (Anupama and Ravindra 2000). 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.) (Becker 2007). A comparison of the nutritional value of algal biomass, MBM and PPB is shown in Table 38.

Table 38: 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

12.13.2 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 (Savage 2011). 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 (Craggs et al. 2013). Currently the most cost effective photo-bioreactor is the open raceway pond (Acién Fernández et al. 2001) with a huge footprint due to shallow water for light penetration and a 10 day HRT (National-Research-Council 2012). 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 (Posten 2009).

Similar to reports about algae, cyanobacteria have been applied for sec (Sawayama et al. 1999) and tertiary sewage treatment (Chevalier et al. 2000) but also for a wide range of industrial wastewaters (Canizares et al. 1993). 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} (Loogman et al. 1980)) and higher affinity to N and P (Ray 2006)) although the general growth rates are slower compared to most microalgae (Talbot and de la Noüe 1993). Some species are photoheterotrophic but the majority rely on CO_2 addition and reported HRTs were several days (Talbot and de la Noüe 1993). 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 (Aráoz et al. 2010). 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 (Aráoz et al. 2010). The occurrence of microtoxins in wastewater has been reported (Vasconcelos and Pereira 2001) and phytoplankton bloom containing elevated levels of microcystin producing *microcystis aeruginosa* are common in wastewater treatment plants (Barrington and Ghadouani 2008). 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.

12.14 **Key challenges and knowledge gaps for application of PPB to red meat wastewater**

Based on more than 3 years of intensive research in other applications, the following challenges are identified for PPB in slaughterhouse applications:

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.

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.

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.

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.

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.

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.

Additional challenges identified for diluted wastewater treatment are not a 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 of 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.