Utilization of microalgae to purify waste streams and production of value added products

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PREPARED BY:	Roberta Fornarelli, Parisa A. Bahri, Navid Moheimani
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GLOSSARY

AMPC: Australian Meat Processor Corporation

AGWR: Australian Guidelines for Water Recycling

AD: Anaerobic Digestion

BOD: Biological Oxygen Demand

COD: Chemical Oxygen Demand

CO_2: carbon dioxide

FOG: Fat, Oil and Grease

HRT: Hydraulic Retention Time

HSCW: Hot Standard Carcass Weight

MLA: Meat and Livestock Australia

NH_4-N: Ammonium as nitrogen

NO_2-N: Nitrite as nitrogen

NO_3-N: Nitrate as nitrogen

PO_4-P: Phosphate as phosphorus

SCOD: Soluble COD

TCOD: Total COD

TDS: Total Dissolved Solids

Temp: Temperature

TKN: Total Kjeldahl Nitrogen

TKP: Total Kjeldahl Phosphorus

TN: Total Nitrogen

TOC: Total Organic Carbon

TP: Total Phosphorus

TS: Total Solids

TSS: Total Suspended Solids

VS: Volatile Solids

1.0 EXECUTIVE SUMMARY

As a substantial consumer of water, the Australian red meat processing industry produces large volumes of wastewater that are rich in nutrients and organic matter. On-site treatment of wastewater is needed to ensure compliance with the existing guidelines for safe discharge and recycle. The majority of Australian abattoirs have wastewater treatment plants on-site, most of which includes anaerobic digestion (AD) as the main biological treatment aiming at reducing the organic content. High concentrations of nitrogen and phosphorous are found in the AD effluents and nutrient removal treatments are not as widely implemented as anaerobic treatments. As a consequence, large volumes of high nutrient content wastewater are dumped in evaporation ponds. Environmental risks of such management practice are associated with greenhouse gas emissions by ammonia volatilization, eutrophication of soils and surface waters due to nutrient imbalance, and groundwater nitrogen contamination. Large land footprint and loss of the environmental and economic value of the wastewater effluent in the form of water and nutrients recovery are major drawbacks of the current management practices. Due to the environmental and economic burden caused by the current wastewater treatment practice, a cost effective and efficient nutrient removal/recovery system is perceived a priority by the red meat processing industry. Waste-to-profit recovery systems that allow the recycle of treated wastewater as well as the production of value added products are considered as a major progress within the industry towards environmental sustainability, economic benefit and process optimization.

Overarching objective of this project is to investigate a new technological approach for the transformation of nutrient contents in red meat processing wastewater streams into protein rich biomass via microalgae cultivation. To address this main objective, a techno-economic feasibility study of an integrated microalgae cultivation process for treating abattoir wastewater effluents and for water recycling has been developed. A thorough literature review has demonstrated the maturity and robustness of microalgae cultivation as a cost-effective treatment technology able to capture nutrient and harvest algae biomass whilst producing a nutrient-depleted water effluent. Through the development of a mathematical model, the output of a microalgae cultivation system integrated on the AD effluents currently generated at two Australian abattoirs has been quantified. The treatment of alternative wastewater streams by microalgae cultivation has been addressed and the effluent from AD was demonstrated as the most suitable stream for integration with the proposed process. As part of the project objectives, the potential for the effluent from microalgae cultivation to be reused and recycled within the abattoir's operations has been addressed through an environmental and risk assessment in the light of current Australian food safety standards.

The proposed microalgae cultivation system generates two product streams: *i)* a concentrated microalgae biomass product and *ii)* a nutrient-depleted water effluent. A microalgae cultivation system that receives 2 ML/d of AD effluent is expected to generate about 1.3 ML/d of nutrient-depleted water suitable for recycle/discharge and about 3 to 5 tons of algae biomass product at 30% solids. At an average concentration of nitrogen and phosphorous in the AD effluent ranging as 150-250 mg/L and 25-35 mg/L, respectively, an 80% to 100% nutrient removal and fixation of 6 to 10 tons/d of carbon dioxide into algae biomass are foreseen. Capital and operational costs are estimated at AUD 2-4M and AUD 1-2M, respectively, for an open raceway pond algae cultivation system followed by settling and centrifugation. Algae unit production costs range within AUD 1.5 to 2 per kg of algae product, thus competitive with the current market price of microalgae sold as animal feed (ranging from AUD 5 to 20 per kg of algae) or fish meal (ranging from AUD 1.5 to 2.15 per kg of algae).

Subjected to water recycling guidelines implemented at each abattoir, the nutrient-depleted water stream can be recycled within the abattoir operations thus improving the environmental impact of the abattoir and reducing costs associated with purchase of freshwater. Suggested uses of the effluent from microalgae cultivation refer to all the processes where non-potable quality water is allowed for use, e.g., cleaning of yards, infrastructures and trucks, washing of animals other than final wash, animal drinking water, fire control, irrigation of gardens and green areas, irrigation of crops and pasture for fodder production. If recycle is not viable, the final effluent is likely to meet the guidelines for safe discharge into the sewer or surface waters. The grown algae biomass represents a process by-product whose intrinsic value can be exploited as a way to offset some treatment costs and possibly produce a revenue stream. On-site use of algae biomass as fertilizer and animal feed are technically feasible and cost effective options. Algae biomass can also be used for producing biogas through AD, thus offsetting some of the abattoir energy demand.

The integrated microalgae cultivation system mitigates, and possibly removes, the environmental impacts associated with contamination of soils, water and groundwater, and with greenhouse gas emissions into the air. More importantly, it gives value to the wastewater effluent by recovering the nutrients and water and ultimately improving the environmental footprint of the abattoir. Several positive impacts of the microalgae cultivation process integrated on the AD effluent are identified:

- Reclamation of the environmental and economic value of the wastewater effluent in terms of water and nutrient recovery;
- Recycle of large volumes of water for on-site and off-site uses;
- Generation of an algae biomass product that is suitable for on-site reuse, energy generation or for sale to available markets;
- Sequestration of carbon dioxide currently generated by AD reactors during the current wastewater treatment process and fixation into algae biomass.

The promising outcomes of this technology assessment pave the road for further and more detailed explorations that aim at improving technical understanding, environmental and economic footprint and potential for full-scale applications. Experimental test works on microalgae cultivation on abattoir wastewaters are deemed essential to corroborate, improve and expand the techno-economic assessment developed in this project. Recommendations for future R&D projects include bioprospecting studies through a pilot plant implemented at the premises of an operating Australian abattoir. Through long-term outdoor experiments, microalgae species suitable for growing on abattoir wastewaters are identified, together with the most cost-effective cultivation and harvesting methods, impacts of seasonal variations of inflow streams and nutrient supplies on algae growth rates. The optimization of microalgae growth conditions for maximum nutrient removal and the evaluation of different microalgae sources for animal/aquaculture feed is also an expected outcome of a bioprospecting study. As highlighted by the sensitivity analysis, it is recommended to carefully address the impacts of algae productivity per unit area and effluent composition (i.e., flow rate and nutrient content) on the overall system performances through extensive experimental test work at pilot scale.

2.0 INTRODUCTION

Australia is amongst the global leaders in the export of red meat and livestock, contributing A$17 billion to Australian economy. The global demand for meat has constantly been increasing, encouraging the industry to develop more sustainable food systems while meeting social responsibilities. In light of more competitive global markets, rising energy prices, and environmental responsibilities, the industry has aimed to improve environmental credentials of their business, while enhancing economic efficiency.

Red meat processing facilities generate large volumes of wastewater and solid waste rich in organic contaminants. Through "waste-to-profit" initiatives and research, these streams can be strong candidates for treatment processes aiming at the recovery of energy and nutrient resources and for the production of value-added products. The proposed project will investigate the integration of microalgae cultivation in the red meat processing facilities as a new process technology towards water treatment, reuse and recycle. The project identifies different sources of water and effluents from solid waste and wastewater treatment in meat processing facilities and evaluates their potential for utilization in a microalgae cultivation process. The cultivation is conducive to purifying the waste streams and production of value added products such as biofuel, cattle feed, and high value pigments. The viability of treated water recycling within meat processing operations is assessed, whilst taking into consideration food safety standards and associated risks. An economic assessment of the proposed system is conducted based on the mathematical model developed in the study. The concept model of the proposed integrated algae cultivation is drafted in Figure 1. Anaerobic digestion (AD) effluents, primarily treated wastewater, raw wastewater, runoff water and alternative water sources generated during meat processing operations are all addressed as potential targets of the proposed microalgae cultivation system. Microalgae have the ability to recover nitrogen and phosphorous from wastewater streams and to sequester the carbon dioxide currently generated by AD processes in abattoirs into algae biomass. The resulting product is clean water and microalgae biomass, both valuable products potentially improving the environmental footprint and economic benefits of the meat processing facility.

This project addresses specific industry needs related to the challenges with the management and treatment of wastewaters produced in abattoirs. The environmental impacts and costs related to the production of large volumes of waters that are high in nutrients, pathogens and organic content represent a critical burden meat processing facilities are currently facing. The application of microalgae cultivation for nutrient removal represents a reliable and mature technology widely applied in the context of wastewater treatment. Moreover, due to the availability of abundant solar resources and land, Australia is considered as one of the best locations for microalgae cultivation systems. Although not yet applied to treat abattoir wastewaters, recent studies conducted at Murdoch University have been successful in growing three high ammonium resistant species of microalgae on undiluted AD piggery effluents. This research represents a proof-of-concept study that illustrated the potential of culturing microalgae in such a very turbid and high ammonium wastewater. To this end, abattoir AD effluents are expected to be a more suitable algae growing substrate than piggery effluents due to lower turbidity and nitrogen content. In the context of red meat processing facilities, the integration of microalgae cultivation system as proposed in Figure 1 is expected to:

- Reduce liquid waste streams, associated treatment and disposal costs by producing a clean wastewater effluent for safe discharge;
- Provide potential for treated wastewater effluents to be reused and recycled within abattoir's operations;
- Reduce the environmental impacts of the abattoir by reducing freshwater consumption, promoting water recycling, reducing the carbon footprint by lowering greenhouse gas emissions;
- Generate additional revenue streams by using the cultivated algae biomass as animal feed, energy source as biogas, fertilizer and high-value pigments;
- Fully integrate a "waste-to-profit" concept through the recovery of valuable resources (i.e., water and nutrients) from wastewaters.

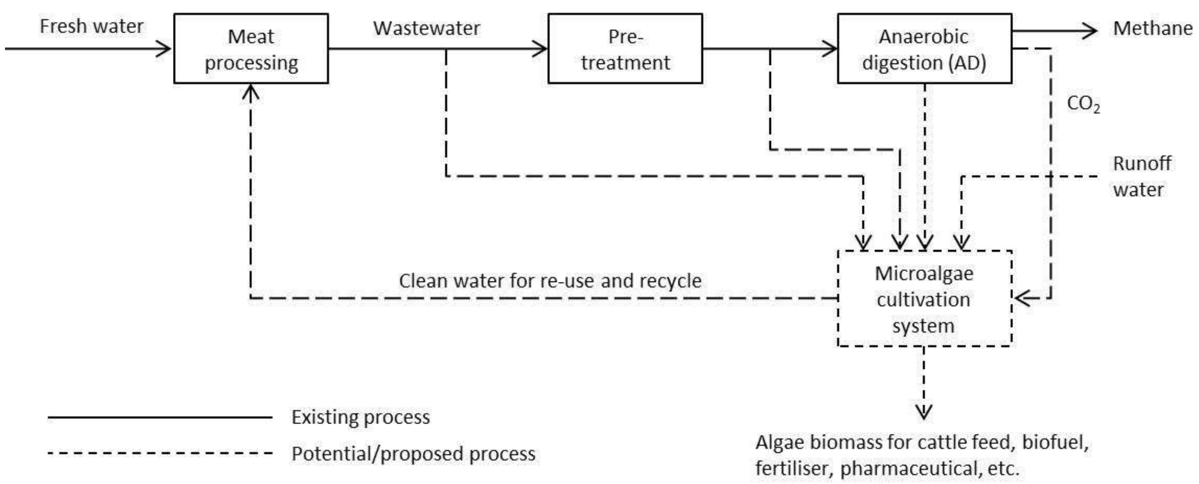


Figure 1. Integration of microalgae cultivation with the wastewater treatment process.

3.0 PROJECT OBJECTIVES

The five overarching project objectives as specified in the signed Agreement are listed below:

1. Identify the potential approaches for the use of alternative water sources (including the effluents from a microalgae cultivation process) in meat processing facilities.
2. Investigate a new technological approach for the transformation of nutrient contents in red meat processing wastewater (and solid waste) streams into protein rich biomass via microalgae cultivation.
3. Identify new potential for water reuse in meat processing facilities (including reuse in the proposed microalgae cultivation system).
4. Investigate new approaches for water recycling (including the algal-treated water recycling opportunities).
5. Assess the environmental and economic performance of the proposed water management

system.

Each objective has been addressed progressively by defining six major project milestones, each focusing on specific project deliverables:

1. Milestone 1. Literature review and data collection
   (i) Review of food safety standards applicable to the meat processing industry
   (ii) Collection of water sources, use, collection, and treatment data from large-scale meat processing facilities for the development of case studies
2. Milestone 2. Literature review on microalgae systems
   (i) Review of microalgae cultivation systems and applications in meat processing industry
   (ii) Review of existing water sources, use, collection, effluent treatment processes including existing anaerobic digestion, runoff water, and nutrient removal systems for comparison with the proposed micro-algal integrated process
3. Milestone 3. Alternative water sources identification and waste water quality assessment
   (i) Characterizing existing water sources, use, and waste collection (based on the potential for the integration of microalgae cultivation process)
   (ii) Identification of alternative water sources
   (iii) Assessment of waste streams quality (nutrient content) and suitability for algae cultivation process.
   (iv) Assessment of flow quantities to be used as input for system sizing.
   (v) Reconfiguration of water/waste water streams considering the integration of microalgae cultivation into the process.
4. Milestone 4. Algae-cultivation system design
   (i) Determination of microalgae cultivation approaches based on wastewater streams identified
   (ii) Development of a mathematical model representing the operation of microalgae cultivation on AD effluents, water runoff and identified water/waste water streams identified (mass and energy balances)
5. Milestone 5. Environmental impact analysis
   (i) Environmental impact assessment (consequential analysis): investigating the environmental performance of the proposed microalgae treatment system in comparison with conventional treatment process used in the sample plant
   (ii) Food safety risk assessment and system enhancement
6. Milestone 6. Cost benefit and uncertainty analysis
   (i) Cost benefit analysis for the proposed microalgae cultivation system
   (ii) Sensitivity and uncertainty analysis

4.0 METHODOLOGY

4.1 Literature review and data collection

As an R&D desktop and modelling project, the review of the published literature produced by the scientific community worldwide in the form of scientific papers, books and conference publications is central to the project methodology. Review of previous and current work developed at Murdoch University (e.g., Ayre et al., 2017; Chaudry et al., 2017; Nwoba et al., 2016; Borowitzka and Moheimani, 2013; Moheimani 2005) and by R&D MLA/AMPC projects (Hamawand et al., 2015; Ridoutt et al., 2015; Jensen and Batstone, 2012; Jensen and Batstone, 2013) constitutes in-house background knowledge and a substantial source of information, particularly in regards to data on Australian abattoirs. About 30 AMPC/MLA reports have been reviewed to achieve the objectives of Milestone 1. Major attention was given to recent reports, published in the last five years, in order to capture the latest trends and innovations achieved by the red meat industry in Australia. The review by Bustillo-Lecompte and Mehrvar (2015) was used as the reference work in regards to the recent trends and advances on the characterization and treatment of abattoir wastewaters worldwide. Information on food safety standards and regulations was collected through personal communication with export-registered abattoirs, MLA and AMPC, the Department of Agriculture and Water Resources, the Department of Health, and the Office of the Environmental Protection Authority.

Two AMPC reports (Jensen and Batstone, 2012; Jensen and Batstone, 2013) and personal communication with the reports' authors have provided access to a large amount of information and data on six Australian abattoirs (hereafter referred to as Sites A to F). Personal communication between Murdoch University and another abattoir (hereafter referred to as Site G) has provided access to the abattoir's data on wastewater production and treatment. The level of detail of the collected data varies for each abattoir depending on data availability, confidentiality agreements, abattoir's operations and routine monitoring policies. An in-depth review of the data available at each site is reported in Appendix 9.1. For the purpose of this project, a comprehensive dataset is available for three abattoirs only, while other abattoirs supplied partial and/or inaccurate information. Out of the seven reviewed abattoirs (Appendix 9.1), Sites A, D and G have been selected as the most appropriate ones to develop the mathematical model as they provide the most comprehensive and reliable set of data. There are substantial differences between Site G dataset and the data available at Sites A and D. Site G data are taken at different sampling points within the abattoir's wastewater treatment plant and are generated by routine monitoring programs over a long-time period (three years of routine monitoring). The data published by Jensen and Batstone (2012, 2013) on Sites A and D are taken at different sampling points within the slaughtering process, as opposed to within the abattoirs' wastewater treatment plant like at Site G, and were measured during a monitoring campaign of intensive sampling lasting a few days (as opposed to the three-year dataset provided by Site G). Available data on Sites A and D thus represent an instantaneous picture of the abattoir's operations rather than its continuous operations. For these reasons, a direct comparison between Site G and Sites A and D is not entirely meaningful and appropriate, and the two sets of abattoirs are presented and analyzed separately.

Major upgrades occurred at Site A and D after the work of Jensen and Batstone (Jensen and Batstone, 2012; Jensen and Batstone, 2013). A new covered lagoon was installed at Site A aiming at collecting and re-using the biogas produced by anaerobic digestion (AD). An upgraded system for blood collection was installed at Site D which contributed to lower the nitrogen concentration in the wastewater by about 30% the value measured by Jensen and Batstone in their 2013 MLA/AMPC report. The data used in the present project refer to the pre-upgrade conditions at Sites A and D. Also, Sites A and D show many similarities in size, location, wastewater composition, handling and treatment. Site A is located in Southern Queensland and Site D is located in Northern New South Wales, with a distance of only about 200 km between the two abattoirs. Both abattoirs are similar in size (about 1,000 heads processed daily) and the composition of the treated wastewater after AD is very similar. Because of these similarities, although both sites have been presented and analyzed separately for most of the project, major attention in terms of mathematical modelling, cost analysis and discussion has been given to Site D. The same data interpretation and process conclusions are applicable to Site A.

4.2 Mass balance of the microalgae cultivation system

The mass and energy balances of the integrated microalgae cultivation process have been developed. Amongst the reviewed Australian abattoirs, three sites have been selected for modelling, namely Sites A, D and G. Those sites have been selected because a comprehensive set of data which includes pre and post AD characterization and wastewater flow rates across the abattoir is available.

The approach used in the calculation of the mass and energy balances is explained hereafter. The values of modelling parameters such as algal productivity and energy consumption rates are taken from recent experimental and modelling studies on microalgae cultivation systems. Note that the modelling parameters related to microalgae dynamics (e.g., growth, settling) refer to experimental values on the cultivation of the microalgae *Chlorella* which has been shown to grow reliably on challenging substrates and conditions (Ayre et al., 2017; Ras et al., 2011). The uncertainty associated with the modelling assumptions and input data represents the main source of error and is addressed by an appropriate sensitivity analysis.

The mass balance of the microalgae cultivation system aims at quantifying the concentrations and mass fluxes of all the components involved in the process. The components involved in the growth of microalgae are water, sunlight, carbon dioxide, and some nutrients, mainly nitrogen and phosphorus. The photosynthetic reaction that takes place in a microalgae cultivation system is described by Eq. 1, where $C_{106}H_{263}O_{110}N_{16}P$ is the approximate chemical formula for microalgae estimated from the Redfield Ratio (Borowitzka and Moheimani, 2013).

$$106CO_2 + 16NO_3^- + H_2PO_4^- + 122H_2O + 17H^+ \xrightarrow{light} C_{106}H_{263}O_{110}N_{16}P + 138O_2 \qquad \text{Eq. 1}$$

The selection of an appropriate wastewater stream to be integrated with a microalgae cultivation system was the specific deliverable of Milestone 3 report (refer to Section 5.3 and Appendix 9.3) and the AD effluent stream resulted to be the most appropriate due to its low concentration of organics and high nutrient content. Based on the composition and flow rate of the AD effluent stream, a daily flux of nitrogen, phosphorus and carbon dioxide is calculated. Whilst the amount of nitrogen and phosphorus available to grow algae is provided by the wastewater itself, the amount of carbon dioxide available to the process is assumed to be recycled from the AD process. It is assumed that the methane produced in the AD pond is burnt (following the combustion reaction described in Eq. 2), and the resulting CO_2 is recycled as input to the microalgae cultivation system. Although the biogas produced by the AD pond at Site G is not currently captured, personal communication with Site G personnel has confirmed on-site methane capturing and combustion as one of the abattoir's short-term priority. In the following mass and energy balance calculations, it is therefore assumed that CO_2 is available at Site G by AD biogas combustion.

$$CH_4 + 2O_2 \longrightarrow CO_2 + 2H_2O \qquad \text{Eq. 2}$$

The three components needed for algal growth, i.e., carbon dioxide, nitrogen and phosphorus, are not expected to be in perfect balance, therefore one component (usually nitrogen or phosphorus) is likely to be limiting the biomass growth. The limiting component is determined by the stoichiometric ratio of each component to one another as well as the theoretical ratio in the Redfield equation, i.e., C:N:P = 106:16:1 (Eq. 1). This ratio is applied to the molar concentrations of each component. Based on the calculation of the limiting component and the Redfield stoichiometry, the theoretical amount of algae biomass that can grow on the selected wastewater stream is calculated. The fractions of nutrients and carbon dioxide that have not been used for algal growth are also calculated as the difference between the incoming flux and the amount of nutrients and carbon dioxide fixed in the algae biomass. Once the theoretical amount of biomass is calculated and assuming a typical productivity of the microalgae cultivation system from literature values (25 g/m^2/d, annual average, Collet et al., 2011; Ayre et al., 2017), the size of the microalgae cultivation system is estimated. After the cultivation phase, the biomass is harvested and dewatered before final use. It is considered that the biomass is harvested on a daily basis at the same rate at which it grows. The harvested stream from the algae pond is highly diluted and a typical algae concentration is considered at 0.5 g/L (0.05% solids, Collet et al., 2011). Natural settling followed by centrifugation can achieve algae concentrations at 30% solids, thus preparing the algae biomass for further dewatering and drying whether the final biomass is to be transported for off-site uses. A 90% efficiency of the dewatering process is considered (Chaudry et al., 2017).

During the cultivation phase in open ponds, a significant amount of water is expected to be lost by evaporation, thus a quantification of the evaporative flux is also required (Eq. 3):

$$F_{EV} = R_{EV} \cdot A \cdot \rho_W \qquad \text{Eq. 3}$$

Where F_{EV} is the evaporative flux (kL/d), R_{EV} is the rate of evaporation (cm/d), A is the area of the cultivation system (m^2), and ρ_W is the density of water (kg/m^3). An amount of water that is equivalent to the volume lost by evaporation is required to be continuously added to the microalgae cultivation system to make up for evaporation losses. The water resulting from the dewatering step is appropriate for recycling into the growth ponds to make up for evaporation losses.

A schematic representation of the mass balance of the microalgae cultivation process is shown in Figure 2. This mass balance represents the base case and generates two product streams (Figure 2): a 30% solids microalgae biomass and a nutrient-depleted water effluent. The values of parameters and constants used in the mass balance calculations are summarized in Table 1. Note that values refer to raceway ponds as the microalgae cultivation system of choice in this project. The choice of raceway open ponds over closed photobioreactors is further discussed in Section 5.4.1.

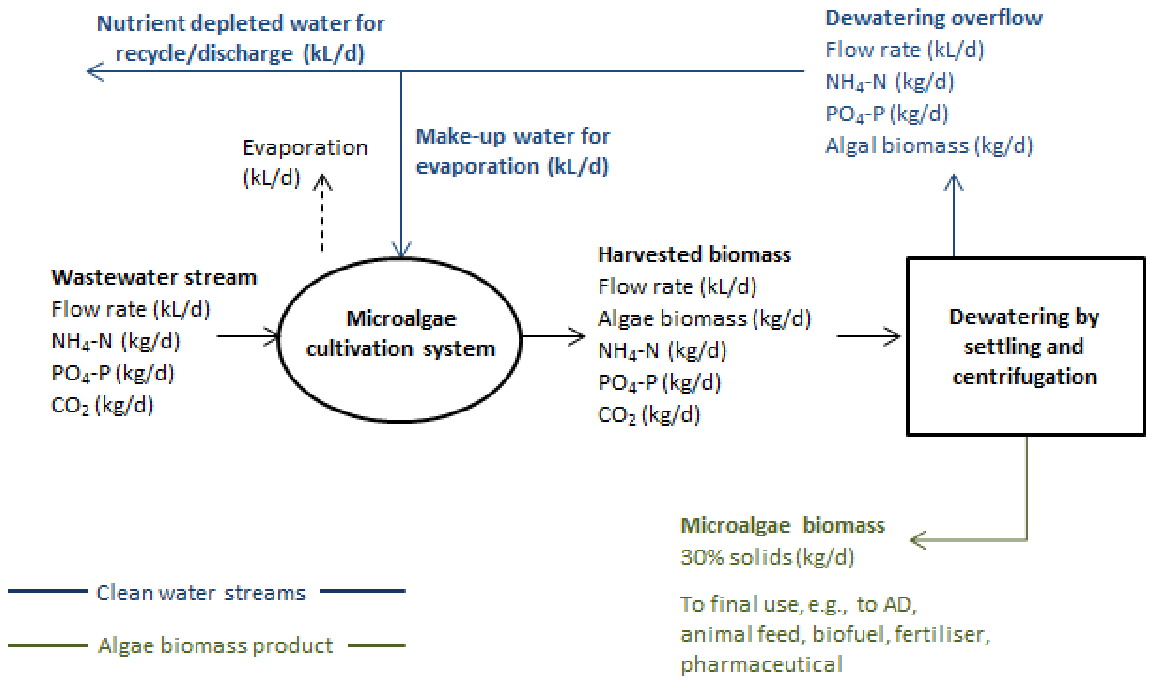


Figure 2. Schematic representation of the mass balance.

4.3 Energy balance of the microalgae cultivation system

The energy balance aims at quantifying the energy inputs and outputs associated with the microalgae cultivation system. The main energy output is related to the amount of energy contained in the dry algal biomass and is quantified by the calorific value of the biomass itself (Table 1). The energy inputs are related to the energy demand of cultivation process, harvesting and dewatering of algae biomass.

The amount of energy required by the cultivation process is most entirely related to the mixing of the system by the paddlewheel mechanism. Its quantification is given by Eq. 4, where E_{CUL} is the energy input to the cultivation system (kWh/d), ER_{PW} is the energy demand by the paddlewheel mixing system (kWh/ha/d), A is the area of the cultivation system (ha), and OD is the operating days (d). Note that, as a strategy to reduce energy consumption, recent experimental tests conducted at Murdoch University have demonstrated the feasibility to operate the paddlewheel mixing mechanism during the day only (12 hours per day) instead of continuous operations. Although a 12 hour/day operation of the paddlewheel mixing process is possible, a 24 hour process is considered hereafter as a high range estimate of energy demand.

$$E_{CUL} = ER_{PW} \cdot A \cdot OD \quad \text{Eq. 4}$$

No energy is required for natural settling of the harvested biomass; however, dewatering by centrifugation at 30% solids is an energy intensive process. Collet et al. (2011) assume an energy demand of the centrifugation step at a loading rate of 7 to 10 g/L of biomass equal to 1 MJ/kg of biomass. Whether a lower solid content is required (i.e., 5% solids), the energy demand decreases to 0.15 MJ/kg of biomass (Collet et al., 2011). The amount of energy required for dewatering is expressed by Eq. 5

$$E_{DEW} = ER_{DEW} \cdot F_{water} \qquad \text{Eq. 5}$$

Where E_{DEW} is the energy required for dewatering (kWh/d), ER_{DEW} is the energy consumption per kilogram of algae biomass to dewater (kWh/kg), and $F_{biomass}$ is the daily flux of harvested biomass (kg/d).

Energy supply is also required for water and carbon dioxide pumping. The values of energy consumption rates suggested by Collet et al. (2011) and Chaudry et al. (2017) have been used in the energy balance (Table 1). Note that the energy required for pumping the algae inoculum to the pond system and the energy associated with wastewater pumping throughout the system are considered negligible comparing to the energy demands of mixing and dewatering. Whether a pilot system is to be designed, the energy rates associated with pumping water and algae streams are to be included in the energy balance.

Table 1. Values of parameters and constants for mass and energy balances.

Parameter/constant	Value	Reference
Mass Balance		
Algal productivity in open ponds (g/m^2/d)	25	Collet et al., 2011; Ayre et al., 2017
Depth of raceway ponds (m)	0.30	Collet et al., 2011
Rate of evaporation R_{EV} (cm/d)	0.4	BoM Australia
Density of water ρ_W (kg/m^3)	1,000	-
Density of biomass $\rho_{biomass}$ (kg/m^3)	1,000	Chaudry et al., 2017
Energy Balance		
Calorific value of 90% solids algae biomass (kWh/kg)	3.5 – 4	Ghayal and Pandya, 2013
Energy demand by the paddlewheel mixing ER_{PW} (kWh/ha/d)	48	Chaudry et al., 2017
Energy demand by dewatering (kWh/kg)	0.04 – 0.3	Collet et al., 2011
Energy demand by pumping water (kWh/m^3)	0.05	Collet et al., 2011
Energy demand by pumping CO_2 (kWh/kgCO_2)	0.021	Chaudry et al., 2017
Operating days per year OD (d)	330	

4.4 Environmental impact assessment

One of the project objectives aims at determining the environmental impact and risk assessment of the integrated microalgae cultivation process in comparison with the wastewater treatment currently implemented at the selected abattoirs. The assessment of risks associated with the identified environmental hazards is related to the composition of the wastewater effluents and algae cultivation products (i.e., nutrient-depleted water effluent and algae biomass) estimated by the mass and energy balances. The uncertainty associated with the calculation of the mass balance is going to impact on the results of the environmental impact assessment. For this reason, the assessment discussed in this project is considered a high-level evaluation of environmental impacts and risks. A full environmental impact assessment integrated with an appropriate life cycle analysis is recommended once an ad-hoc experimental campaign provides more data and understanding of the integrated microalgae cultivation process (e.g., bio-prospecting study).

A qualitative characterization of the environmental impacts and associated risks is determined by following the procedure described in Chapter 4 of the Australian Guidelines for Water Recycling, hereafter referred to as AGWR (2006). Table 2.7 of the AGWR (2006) is used to estimate the risk by defining likelihood and consequence of each identified environmental hazard. The main environmental endpoints are combined with the key hazards, the intended uses of the wastewater effluent and their identified environmental impacts to determine the severity of the associated environmental risks. A 'low' risk is defined as acceptable by the AGWR and does not require preventative measures. 'Moderate' to 'Very High' risks require intervention to reduce the risk levels to acceptable. In this study, major attention is given to hazards caused by high concentrations of nitrogen and phosphorous when the treated abattoir wastewater is discharged into storage ponds and/or recycled for the on-site and off-site uses permitted by the AGWR (e.g., irrigation, fire control, and wash down of tracks and yards). Nitrogen and phosphorus are included in the nine key environmental hazards to be considered in risk assessment studies when the treated wastewater is in contact with environmental endpoints such as soils, surface waters and groundwater (AGWR, 2006). The concentration of nutrients in the final wastewater effluent is the main target of the proposed microalgae cultivation system when compared to traditional wastewater treatment process, thus making the focus on nutrient concentrations particularly significant for the current project. Other key environmental hazards to be analyzed in a full environmental impact assessment include boron, cadmium, chlorine disinfection residuals, hydraulic loading, salinity, chloride, sodium, and microbial content (AGWR, 2006). Together with nutrient concentrations, those hazards will determine the suitability of the final effluent to be recycled in the abattoir's operations.

4.5 Cost-benefit analysis of the microalgae cultivation system

The report by worldwide renowned algae expert John Benemann (Lundquist et al., 2010) on the economics of microalgae cultivation in open raceway ponds is used as the main reference for the cost-benefit analysis developed in this project. This report is a highly detailed economic analysis of a microalgae cultivation system coupled with a wastewater treatment plant to treat wastewaters produced by farming and agriculture activities. Similar to our current application of microalgae to treat abattoir's wastewaters, the emphasis is on the wastewater treatment side with the algae biomass being a valuable by-product. The unit costs reported by Lundquist et al. (2010) are defined per hectare and refer to 2010 US dollars. An AU to US dollars conversion rate of 1.3 and an US inflation rate of 1.12 (based on 2017 and 2010 Consumer Price Index equal to 244 and 218, respectively, CPI website) are considered to convert 2010 US dollars into 2017 AU dollars. Costs associated with inoculum cultivation, labor, engineering fees, contingencies and taxes are sourced from Wijihastuti (2017) and are representative of 2017 Australian conditions. All unit costs used in the cost-benefit analysis are summarized in Table 2.

Table 2. Summary of capital and operational costs.

	Value	Unit	Reference
Capital Costs			
Open raceway pond – clay lined	34,100	2010 US$ / ha	Lundquist et al., 2010
Buildings, roads, drainage, vehicles	4,610	2010 US$ / ha	Lundquist et al., 2010
Electrical	19,000	2010 US$ / ha	Lundquist et al., 2010
Water piping	14,000	2010 US$ / ha	Lundquist et al., 2010
CO_2 (flue gas+ distribution)	5,940	2010 US$ / ha	Lundquist et al., 2010
Dewatering system	12,130	2010 US$ / ha	Lundquist et al., 2010
Digesters	21,900	2010 US$ / ha	Lundquist et al., 2010
Biogas turbine	24,400	2010 US$ / ha	Lundquist et al., 2010
Inoculum system	15,996	2017 AU$	Wijihastuti, 2017
Standardized indirect capital costs			
Engineering fees	15% of capital	%	Wijihastuti, 2017
Contingency	5% of capital	%	Wijihastuti, 2017
Working capital	5% of total capital	%	Wijihastuti, 2017
Operational costs			
Electricity	0.27	AU$/kWh	
Labour - Plant Manager	113,520	2017 AU$/year	Wijihastuti, 2017
Labour - Engineer	84,480	2017 AU$/year	Wijihastuti, 2017
Labour - Lab Analyst	63,360	2017 AU$/year	Wijihastuti, 2017
Labour - Administration	60,720	2017 AU$/year	Wijihastuti, 2017
Labour - Technician/Pond Operator	50,160	2017 AU$/year	Wijihastuti, 2017
Maintenance/ Insurance	10% of total capital	%	Wijihastuti, 2017
Tax	27.5% of total capital	%	Wijihastuti, 2017

4.6 Sensitivity and uncertainty analysis

A sensitivity analysis was conducted to identify how the uncertainty associated with the modelling assumptions can impact the system's performance. The modelling assumptions subjected to sensitivity analysis are related to the characterization of the AD effluent (i.e., nutrients concentration and AD effluent flow rate), algae productivity per unit area, specific energy requirement for pond mixing and dewatering as the main energy demanding processes, capital costs associated with pond construction, harvesting system and other capital costs lumped together (buildings, roads, water piping, electrical system). The system's performance affected by the modelling assumptions is related to the daily amount of harvested biomass, the daily flow rate of recycled nutrient-depleted water, algal pond size, total capex and opex, and algae production unit costs.

Table 3 summarizes the base case values assumed in the mass and energy balance calculations and the deviation from base case as evaluated in the sensitivity analysis. A 50% variation at 25% increments has been chosen for the concentration of nutrient and flow rate in order to capture the variability observed at the different sites. The three-year dataset provided by Site G has shown a ± 25% variation from the average concentration of total nitrogen and phosphorus, thus the sensitivity analysis at ± 50% is expected to cover the wastewater composition variability as measured on site. A greater than 50% variation of the flow rate could occur at Site G; however, the absence of historical flow rate data due to a poor monitoring system does not allow a more specific estimate of the post AD wastewater stream. The 50% variation of algae productivity takes into account the seasonal variability of algae growth, with summer peaks at 40 $g/m^2/d$ and winter minima lower than 10 $g/m^2/d$. Opex and capex have been varied from -25% to +100% at 25% increments to take into account the large variability of the modelling assumptions related to the system's costs.

Table 3. Base-case values and percentage variation of the modelling parameters tested by sensitivity analyses.

Modelling parameter	Base case	Variation from base case (%)
Wastewater characterisation		
Nitrogen concentration (mg/L)	Site D: 229; Site G: 151	-50; -25; +25; +50
Phosphorous concentration (mg/L)	Site D: 32; Site G: 26	-50; -25; +25; +50
AD effluent flow rate (kL/d)	Site D: 2,150; Site G: 1,750	-50; -25; +25; +50
Algae growth		
Algae productivity ($g/m^2/d$)	25	-50; -25; +25; +50
Operational costs		
Specific energy for pond mixing (kWh/ha/d)	48	-25; +25
Specific energy for dewatering (kWh/kg)	0.3	-25; +25
Capital Costs		
Open raceway pond – clay lined (2017 AU$ / ha)	49,620	+25; +50; +100
Dewatering system (2017 AU$ / ha)	17,651	+25; +50; +100
Others (2017 AU$ / ha)	79,367	+25; +50; +100

5.0 PROJECT OUTCOMES

5.1 Milestone 1. Literature review and data collection

5.1.1 The red meat processing industry and its environmental impacts

The red meat processing industry in Australia comprises of around 191 sites (i.e., individual facilities) spread across 120 businesses (Hamawand, 2015). A process flow diagram of the operations that typically occur in abattoirs is summarized in Figure 3 (COWI, 2000). Cattle are delivered to the abattoir in trucks and unloaded into holding pens, where they are washed before slaughter. After stunning, bleeding takes place with the blood being collected in a trough for disposal or for further processing. The bled carcasses are conveyed to the slaughter hall where dressing and evisceration take place. Once the edible and inedible offal are separated and the carcass has been washed, it is sent to a cold storage area for rapid chilling. Carcass cutting and boning often take place after chilling and a following inspection of carcasses and viscera ensure they are suitable for human consumption. At various stages in the process, inedible by-products such as bone, fat, heads, hair and condemned offal are generated. These materials are sent to a rendering plant either on site or off site for rendering into feed materials. Of the average 600 kg live cattle weight, about 45% becomes the final meat product, another 30% is inedible material for rendering (bone, fat, heads, hair and condemned offal), 8% and 6% are hide and edible offal, respectively, 7% is blood and the remaining 4% is constituted by paunch manure, blood loss and other losses (COWI, 2000; Jensen and Batstone, 2012).

Red meat processing facilities critically depend on water and energy for their operations. The average total energy consumption of an abattoir is about 3,000 MJ per ton of hot standard carcass weight (tHSCW) as reported by the 2015 AMPC environmental performance review report (Ridoutt et al., 2015). High level of thermal energy in the form of steam and hot water is routinely used for cleaning, sanitizing and rendering. Approximately 80-85% of the total energy need of an abattoir is for thermal energy, with the remaining being electricity used for the operation of machinery and for refrigeration, ventilation, lighting and the production of compressed air (COWI, 2000). Rendering operations are normally the largest consumer of energy.

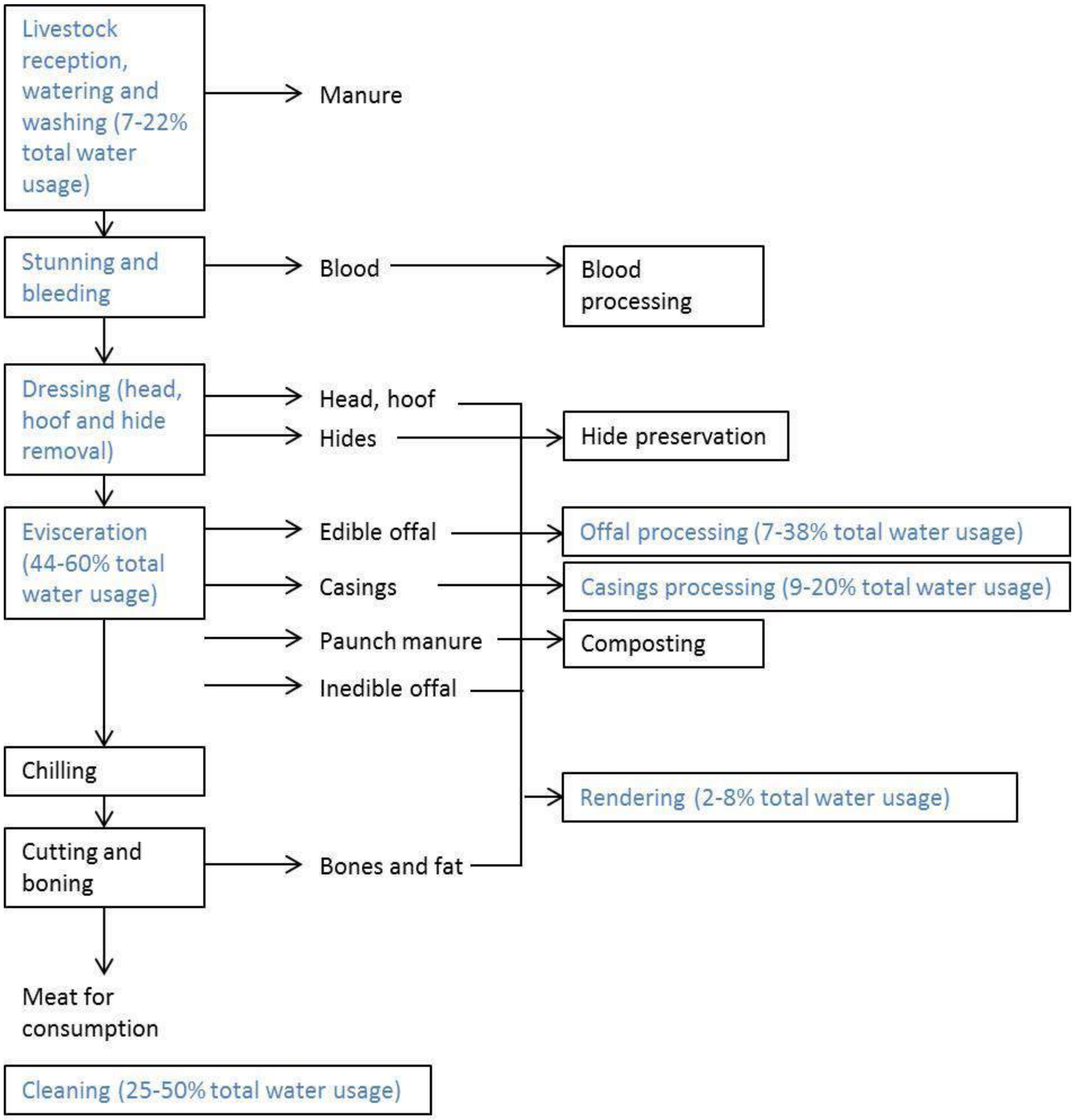


Figure 3. Process flow diagram of red meat processing operations. Operations with a substantial water consumption rate are highlighted in blue (COWI, 2000).

Within the food and beverages industry, the meat processing industry is the largest consumer of water (Bustillo-Lecompte and Mehrvar, 2015). Water is mainly used for watering and washing of livestock, washing of animal products (e.g., casings, offal, and carcasses), washing yards, trucks, unloading areas and stock floors, cleaning and sanitizing of equipment and processing areas. The operations that require large water volumes and the partitioning of water consumption are highlighted in Figure 3. In their book on meat processing, COWI (2000) refers to a water consumption that ranges from 4 to 15 kL/tHSCW. The World Bank Group states that a slaughterhouse plant can consume between 2.5 and 40 kL of water per metric tons of meat produced (Bustillo-Lecompte and Mehrvar, 2015). Following the adoption of new protocols and technologies that promote water use efficiency and water recycling programs (e.g., weekly benchmarking of site water use efficiency, reuse of sterilizer water, installed additional water meters and timers to better understand water flows, use of recycled water for lawns, washing cattle, cleaning yards and screens), the consumption of water in Australian processing plants has reduced significantly. From 16.6 kL/tHSCW in 1978, water consumption in abattoirs reduced to a range from 2.8 kL/tHSCW for small plants processing less than 1,500 tons of HSCW per week, to 8.6 kL/tHSCW for large export-registered plants (Ridoutt et al., 2015; Hamawand, 2015). In their annual performance review of the 2013/2014 operations, AMPC confirmed a water intake varying between sites from 5.7 to 12.7 kL/tHSCW cattle equivalent. The water consumption is indicatively partitioned as follows (Ford, 2013): 25% of the total water consumption is used for slaughtering, 22% for plant cleaning, 20% for stockyards and trucks washing, 13% for render and service, 10% for sterilizing, and 10% for paunch, gut and offal processing. Town water is the most important source of water intake, followed by bore water, local dams, direct withdrawal from a river, and rooftop rainwater harvesting as minor sources. Out of the 14 facilities reviewed by AMPC in a 2013-2014 survey, 13% of the water demand was met by recycled water as 5 of the 14 sites reported using recycled water (Ridoutt et al., 2015). The source of recycled water is commonly the treated wastewater effluent. Depending on the final use of the recycled water, the stage at which wastewater is treated varies from pre-treatments by screening and floatation to more advanced secondary and tertiary treatments, until possibly achieving potable standards. The most common uses of recycled water consist in on-site and off-site irrigation, watering gardens, flushing toilets, and washing down external areas.

The red meat processing industry is a substantial producer of wastewater streams that are rich in nutrients and organic matter. The average site wastewater production has been reported as 8.5 kL/tHSCW (Ridoutt et al., 2015), which is nearly as high as the average water intake of 8.6 kL/tHSCW (Ridoutt et al., 2015). Although some water is held up with by-products and/or lost through evaporation, local rainfall is an additional source of water that adds to the water balance. It should be noted that the quality of wastewater is highly dependent on the presence of on-site rendering activities: although rendering only consumes about 5% of the total water requirements, the organic strength of the generated wastewater is extremely high and contributes to about 60% of the total organic load. Treatment of wastewater is almost always needed to ensure compliance with the existing guidelines for safe discharge into sewer and/or water bodies and on-site recycling.

5.1.2 Wastewater characterization and treatment

The organic content of wastewater generated by Australian facilities is extremely high (5,000-10,000 mg/L COD) with nitrogen levels generally at 5% of COD concentrations and solids representing approximately 70% of the total COD (Jensen and Batstone, 2012; Jensen and Batstone, 2013). Slaughterhouse wastewater characterization as reported in recent review papers (Jensen and Batstone, 2012; Bustillo-Lecompte and Mehrvar, 2015) is summarized in Table 4. The composition is indicative and it can vary considerably: a concentration of Fat, Oil and Grease (FOG) as high as 1,780 mg/L has been reported by AMPC in a recent annual report (Ridoutt et al., 2015).

Of the seven abattoirs reviewed in this project (hereafter referred to as Sites A to G, Appendix 9.1), Table 5 summarizes the composition of the combined wastewater at each study site. In general the concentration (and load) of organic compounds are greater, or at the higher range, than the reported literature values, while nutrient values are within the range of literature data. The consumption of water varies across the sites and it is consistent with the estimate given in the latest AMPC report (Ridoutt et al., 2015) equal to 8.6 kL/tHSCW. Water consumption at Site G is based on an estimate expressed as kL of water per head per day, as the exact measurement is unavailable (personal communication with the abattoir's operator). A detailed review of each abattoir's process flowsheet, wastewater treatment process and available data is given in Appendix 9.1.

Table 4. Characterization of wastewater generated by red meat processing operations.

	Concentration (mg/L)[a]	Load (kg/tHSCW)[b]	Load (kg/head)[b]	Load (kg/t live)[b]
COD	500 – 15,900	16.7 – 44.4	6 – 16	10 – 26.7
BOD	150 – 4,635			
TSS	270 – 6,400	9.3 – 22.2	3 – 8	5 – 13.3
TN	50 – 841	1.4 – 4.2	0.5 – 1.5	0.8 – 2.5
TP	25 – 200	0.1 - 0.4	0.05 – 0.15	0.1 – 0.3
FOG	270 – 1,800[c]	2.8 – 13.9	1 – 5	1.7 – 8.3
pH	4.90 – 8.10			

[a] Bustillo-Lecompte and Mehrvar, 2015

[b] Jensen and Batstone, 2012

[c] COWI, 2000; Ridoutt et al., 2015

Table 5. Characterization of wastewater at the reviewed abattoirs, and comparison with literature data. NA: not available data.

Site (capacity)	Water (kL/tHSCW)	COD (mg/L)	TS (mg/L)	FOG (mg/L)	TN (mg/L)	TP (mg/L)
Literature	5.6-22.2	500-15,900	270-6,400[1]	100-600	50-841	25-200
Site A (800-1,200 heads/d)	8.1	12,893	8,396	2332	245	53
Site B (NA)	7.4	9,587	4,300	783	232	50
Site C (NA)	14.7	10,800	7,530	3350	260	30
Site D (800-1,400 heads/d)	11	12,460	7,400	1500	438	56
Site E (3,000 heads/wk)	7.1	10,925	6,118	1569	272	47
Site F (NA)	7.1	7,170	3,806	1915	182	27
Site G (500 heads/d)	2-5 kL/head/d	587 ± 440 (as BOD)	1,652 ± 1,011[1]	395 ± 413	123 ± 62	25 ± 14

[1] TSS measurement

The treatment of abattoir wastewaters comprises a series of processes that aim at reducing the concentration of organics, nutrients and pathogens to levels that are acceptable for discharge into sewer or freshwater bodies. Wastewaters are commonly pre-treated by screening, settling, blood collection, and fat separation, followed by physicochemical treatments, including dissolved air floatation (DAF) and coagulation/flocculation, aiming at removing FOG and solids. Secondary biological treatments (aerobic and/or anaerobic processes) aim at reducing the soluble COD and nutrient concentrations. Although the organic matter and nutrient removal can achieve high efficiencies, further treatment by membrane technologies and advanced oxidation processes are normally required to achieve strict discharge guidelines. A summary of the most common wastewater treatment processes used by the meat processing industry is shown in Figure 4 (Bustillo-Lecompte and Mehrvar, 2015).

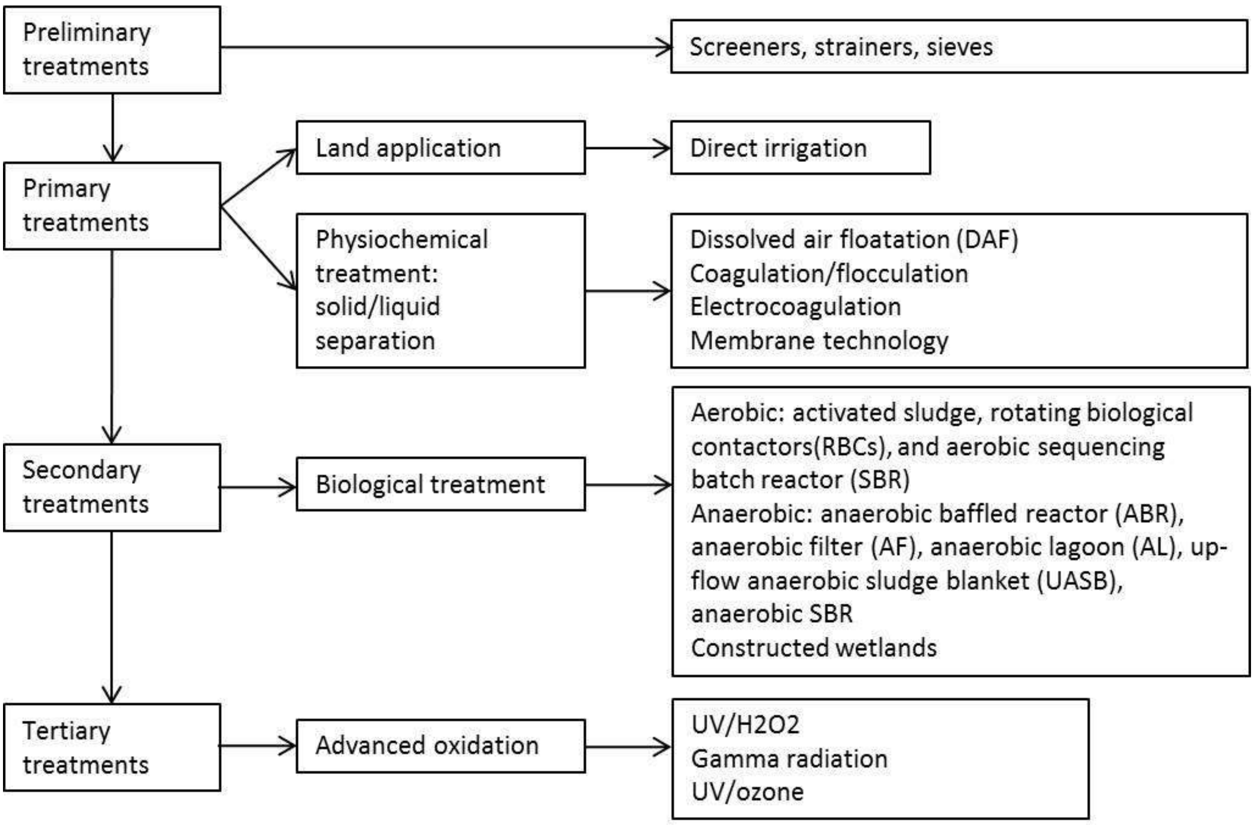


Figure 4. Abattoir wastewater treatment process.

The following processes are the most conventional ones found in the reviewed abattoirs Sites A to G:

- Preliminary treatments: solids screening. Solids are normally separated from the wastewater and sent to compost.
- Primary treatments: separation of solids and fats by saveall/DAF systems, where fats, oils and grease are collected from the surface, whilst solids accumulate at the bottom.
- Secondary treatments: AD is extensively used to reduce the organic load of the wastewater. Soluble organic materials are converted to volatile acids, carbon dioxide, hydrogen and bacteria cells during anaerobic processes. Volatile acids (mostly acetic acid) and other products are then converted to methane and carbon dioxide by methane-producing bacteria. During this stage, nitrogen is released as ammonium nitrogen, thus the nutrient removal by AD does not often achieve nutrient concentrations within regulation limits.

- Secondary treatments: aerobic treatments based on denitrification-nitrification for nitrogen removal are used to reduce the organic as well as nitrogen load. Organic load is converted to bacterial biomass, whilst ammonium nitrogen is converted to nitrate and to gaseous nitrogen by heterotrophic bacteria.

Table 6 summarizes the efficiency of different processes (mostly anaerobic and aerobic processes) for the removal of COD, BOD and total nitrogen from abattoir wastewaters. Table 6 originates from the table published by Bustillo-Lecompte and Mehrvar (2015) and is adjusted to reflect those wastewaters characterized by COD concentrations representative of Australian meat processing plants. The treatment efficiency of abattoirs wastewaters varies extensively; it depends on several factors including, but not limited to, the characteristics of the wastewater, the hydraulic retention time, and the pollutant concentration in the influent. Combined processes (e.g., anaerobic-aerobic reactors, aerobic-membrane technology, coagulation/adsorption processes) are able to couple the benefits of different technologies and have evolved into a reliable technology able to remove organic compounds as well as nutrients. The selection of a specific treatment mainly depends on the characteristics of the wastewater being treated, the best available technology, and the compliance with current regulations under different political jurisdictions.

Table 6. Comparison of different processes for abattoir wastewater treatment (Bustillo-Lecompte and Mehrvar, 2015, and reference therein).

Processes[1]	HRT[2]	COD[3] (mg/L)	BOD[3] (mg/L)	TN[3] (mg/L)	COD removal (%)	BOD removal (%)	TN removal (%)
AnaP	30-80	7,148-20,400	3,501-8,030		62-96	94	
AnaP	24-48	7,083		547	94		
AnaP	60	4,200-9,100		565-785	72-99		46-64
AnaP	30-97	8,450-41,900	21,000		19-57		
AnaP	39-72	1,040-24,200		296-690	30		
AnaP	24-36	2,273-20,073		570-1,603	51-72		3.5-22
AnaP	46-72	12,000-15,800			60		
AnaP	48-72	1,014-12,100	1,410-7,020		84	94	
AnaP	60-96	5,659-9,238	5,571-6,288		92-97	98-99	
AeP	42	6,400-8,320		260-306	95		97
AeP		24,000	1,198	139	90		
AeP	23	5,590-11,750	3,450-4,365	214-256	74-94		
AeP	29	9,040	5,242		89	90	
CC	-	10,226-15,038	5,042-8,320		32-64	35-68	
AnaP-MF	48-168	2,084-13,381		108-295	97-99		78-90
AnaP-AeP-CC	16-72	6,363-11,000	5,143-8,360	47-138	50-97	98-99	73-93
AnaP-AeP	24	6,000-14,500		300-1,000	99		46

[1] AnaP, anaerobic process; AeP, aerobic process; CC, chemical coagulation; MF, membrane microfiltration

[2] HRT, hydraulic retention time

[3] Influent concentration of COD, BOD and total nitrogen

5.1.3 Food safety standards applicable to the meat processing industry

Food Standards Australia New Zealand (FSANZ) has developed a national food safety standard that covers food safety management in the primary production and processing stages of the meat supply chain. The meat processing is defined as a series of activities that include the admission of animals for slaughter, dressing, boning, packing and production of meat and meat products. All States and Territories have legislation that requires businesses operating abattoirs to be licensed or accredited and to operate in accordance with approved systems to manage meat safety and suitability. The safety of meat and meat products in Australia is currently implemented through reference to the following Australian Standards (AS):

- AS4696-2007 Hygienic Production and Transportation of Meat and Meat Products for Human Consumption
- AS 4466-1998 Hygienic Production of Rabbit Meat for Human Consumption
- AS 4467-1998 Hygienic Production of Crocodile Meat for Human Consumption
- AS 5010-2001 Hygienic Production of Ratite Meat for Human Consumption
- AS 4464-2007 Hygienic Production of Wild Game Meat for Human Consumption

In addition to the standards listed above, individual State Government Departments have set their own State Food Health Acts that apply to the local markets of meat products. If meat products are exported overseas, rules set by Quarantine and Export Control Meat and Meat Products Orders 2005 also apply. A list of the standards reviewed by the authors is summarized in Table 7.

Table 7. Food safety standards applicable to the meat processing industry.

Title	Abbr.
Australia wide	
• Primary production and processing standard for meat and meat products - Standard 4.2.3 - as of 31 July 2015	FSANZ, 2015a
• Proposal P1014 - Primary Production & Processing Standard for Meat & Meat Products	FSANZ, 2015b
• Australian standards for the hygienic production and transportation of meat and meat products for human consumption AS4696-2007	Browne, 2007
• Export Control (Meat and Meat Products) Orders 2005 - as of 1 Sep 2014	Export Orders, 2005
• AQIS Meat Notice 2008/06: Efficient use of water in export meat establishments	AQIS, 2008
Western Australia	
• Food Act 2008 - as of 25 July 2016	Food Act, 2008

The Australian Standards AS4696 2007 (Browne, 2007) set the guidelines in relation to the quality of the water used in the meat processing industry. The standards apply to the water withdrawn from external water sources (e.g., municipal water supply reticulation) as well as to the water that is recycled and reused after on-site wastewater treatment. The standard AS4696 2007 (Browne, 2007) states that only potable water is permitted in all those activities that involve the direct contact with the meat and meat products. Potable water needs to be sourced from safe potable water supply reticulation systems that are protected from seepage from drains, sewerage, septic systems, manure pits and other sources of contamination. The use of non-potable water is allowed in all those circumstances where there is no risk of the water coming into contact with meat and meat products, such as steam production, fire control, the cleaning of yards and the washing of animals other than the final wash.

The use of recycled water by the meat processing industry has been seen as an effective way to reduce fresh water consumption in meat processing facilities and operations, thus decreasing costs relative to freshwater purchase and improve the industry's environmental footprint. Recycled water can be sourced externally; however it is more common to re-use the recycled water that is produced in-house. To this end, the treated wastewater effluent is the most common source of recycled water. In their review on Australian regulations for water recycling in the meat industry, CSIRO in collaboration with AMPC and MLA found only little information on the safety standards regulating the use of recycled water in meat processing facilities (CSIRO, 2014). This is attributed to loading rates that are difficult to be assessed in the guidelines and local soil and climatic conditions needing local assessment. In summary, the following guidelines apply to the use of recycled water in abattoirs:

- Any water and recycled water used in the operations of the facility is to be kept away from human or animal faecal contamination, or needs to be treated to an extent that the risk from human sourced pathogens and chemical contaminants is controlled.
- If recycled water is to be used in meat processing operations, then it must be treated to potable standards as required by the Australian Standards AS4696 2007 (Browne, 2007) and be subjected to the risk assessment procedures Hazard Analysis Critical Control Point (HACCP). The Australian Drinking Water Guidelines (NHMRC, 2011) are considered the document of reference. Although treated to potable standards, the recycled water must not be used as a direct ingredient in meat products or for drinking water at the processing facility (AQIS Meat Notice, 2008).
- Non-potable recycled water can be used for all purposes that do not involve meat productions (e.g., irrigation, pre-wash of cattle and slaughter yard, fire control, the cleaning of yards and the washing of animals other than the final wash, flushing toilets and watering gardens). In this case, the Australian Guidelines for Water Recycling (AGWR, 2006) are considered the reference guideline.
- Non-potable recycled water can be used as livestock drinking water for consumption by cattle above 12 months of age, as defined by the AGWR (2006). In this case, additional water quality objectives should ensure soluble BOD below 20 mg/L, suspended solids below 30 mg/L, *E. Coli* less than 100 CFU (colony forming units) per 100 mL.
- Potable and non-potable recycled water may be used in export registered meat establishments; however, processors must be aware that some countries, such as US, Middle East and Asia, do not permit the import of meat or meat products from processing plants where recycled water has been used anywhere in the plant. The export of meat products is regulated by the Department of Agriculture and Water Resources, which uses the 2008 AQIS Meat Notice, Export

Orders and the Food Standards Australia New Zealand to assess the potential use of recycled water in export registered meat facilities. Water recycling and reuse proposals are currently considered on a case by case basis.

- Direct reuse of water for irrigation is possible with minimal treatment as nutrients and salt levels were often found to be within acceptable limits (AGWR, 2006). However, information on loading rates and impact on local conditions both require consideration to prevent environmental impacts.

When not recycled within the plant's operations, treated wastewater effluents are normally discharged into sewer and/or external water bodies. The minimum requirements for the discharge into sewer and surface water are summarized in Table 8 (ANZECC, 2000; Hamawand, 2015). BOD concentrations into surface water should be lower than 6 mg/L which equates to BOD removal of 99.9% for an initial feed concentration of 4,000 mg BOD/L. A substantial source of contamination is addition of surfactants as a result of the cleaning process. The limit for anionic detergent is set at 0.5 mg/L for drinking water and up to 1.0 mg/L for other purposes water (Hamawand, 2015).

Table 8. Guidelines for wastewater disposal into sewer and surface waters.

Disposal method	Pollutant concentration limit (mg/L)				
	TSS	BOD	COD	TN	FOG
Sewer	1,000-1,500	300-3,000	3 x BOD	-	50-200
Surface water	10-15	5-10	3 x BOD	0.1-15	2-15

5.2 Milestone 2. An integrated microalgae cultivation system for the red meat processing industry

In this project we propose the integration of microalgae cultivation with the treatment of the wastewaters generated in abattoirs as represented in Figure 1. Anaerobic and aerobic treatments are commonly used in abattoirs to reduce the organic and nutrient levels in the abattoir wastewaters. However, some major limitations exist for both treatments. Although anaerobic treatments efficiently remove organic matters whilst producing biogas, nutrient removal is not effectively performed and high nitrogen and phosphorus concentrations are found in AD effluents. Similarly, a large range of nutrients concentration measured in AD piggery effluents is reported in the literature: values range from 1,198 to 3,630 mg/L and from 100 to 600 mg/L for ammonia and phosphorus, respectively (Ayre, 2013). Aerobic treatments are well known for efficiently removing organic matter as well as nutrients; however, their high energy requirement makes these methods expensive. Most importantly, aerobic treatments target nutrient removal other than nutrient recovery, thus not considering potential recovery of valuable resources.

The use of microalgae for the removal of organic contaminants and nutrients from wastewaters is referred to as phycoremediation (Benemann et al., 1977; Cuellar-Bermudez et al., 2017). Microalgae comprise a large group of autotrophic microorganisms with cells composed of proteins, carbohydrates, lipids, fatty acids, pigments, vitamins, and enzymes that can have value for human use (Cuellar-Bermudez et al., 2017). Extensive research started to flourish in the eighties and more so in the last decade as microalgal culture growth has shown great potential in applications not only limited to wastewater treatment and nutrient reduction, but also to produce biofuel, food supplements, protein-rich animal feed, fertilizer for crops and pharmaceutical products (Oswald, 2003). Coupling the treatment of wastewater with the cultivation of microalgae is seen a win–win strategy for both pollution control and biofuel production (Craggs et al., 2013; Zhang et al., 2016): the wastewater represents a continuous and abundant source of water and nutrients needed for algal growth, whilst algae cultivation improves the treatment of wastewater by removing organic pollutants and reducing nutrient concentration. An extensive review on microalgae cultivation recent advances and its integration with slaughterhouse wastewaters is summarized in Appendix 9.2.

As a large producer of wastewater, the food industry has investigated the applicability of microalgae cultivation systems to enhance wastewater treatment. There are two typical applications of microalgae cultivation systems that have been mostly applied to treat piggery wastewaters:

- The microalgae cultivation process is used as a substitute of secondary biological treatments (e.g., aerobic and anaerobic processes). The substrate used to grow microalgae is the primarily treated wastewater effluent which is high in organics and nutrients. The algae biomass is mostly used to produce biofuel, e.g., biodiesel or bioethanol (Fallowfield and Garrett, 1985; Zhu et al., 2013; Maroneze et al., 2014).
- The microalgae cultivation process is applied on the AD effluent which is depleted in organics but still high in nutrients. The algal biomass is recycled back to the AD process to enhance methane production or used for by-products, e.g., biofuel and animal feed. The integration of the AD process and microalgae cultivation have shown to improve discharge effluent quality and methane yield (Hernández et al., 2013; Molinuevo-Salces et al., 2016).

The most relevant studies on the treatment of piggeries and abattoir wastewaters by microalgae cultivation systems are summarized in Appendix 9.2, Tables A2.1 and A2.2. The integration of microalgae cultivation and AD processes has received increasing interest as it offers an economically and technologically attractive solution to the management and post-treatment of AD effluents, normally very high in both nitrogen and phosphorous concentrations (Cai et al., 2013). The Algae R&D Centre at Murdoch University has strong capability in the application of microalgae cultivation systems to AD effluents (Ayre, 2013; Borowtizka and Moheimani, 2013; Nwoba et al., 2016). The work by Ayre (2013) suggests the use of the harvested biomass as either food source to enhance pig production or as a biomass to enrich the AD process. Alternatively, algae biomass can also be used as a crop fertilizer. The harvest of microalgae on AD effluents has been shown as a positive impact on the operation costs and environmental footprint of Australian piggeries (Ayre, 2003).

5.3 Milestone 3. Alternative water sources identification and wastewater quality assessment

The objective of Milestone 3 is to characterize the wastewater streams generated at the reviewed Australian abattoirs, assess them based on their suitability to be integrated with a microalgae cultivation process, and propose a reconfiguration of the current wastewater treatment flowsheets. The full analysis is reported in Appendix 9.3.

Theory and experimental works on phycoremediation techniques have shown that an adequate substrate for microalgae to grow needs to be low in organics (e.g., COD, BOD, FOG) and high in nitrogen and phosphorus content. In general, the optimal wastewater stream to be integrated with a microalgae cultivation process is the AD effluent which is characterized by a low organic and high nutrient content. The low concentrations of sugar and organics found in AD effluents minimize the presence of bacteria and selectively favor the growth of microalgae species. Extensive research conducted at Murdoch University and the study by Wang et al. (2016) suggest the use of undiluted anaerobically treated effluents as optimal candidates for microalgae growth. The experimental work of Maroneze et al. (2014), Taskan (2016) and Hernandez et al. (2016) have shown successful growth of microalgae on primarily treated abattoir wastewaters (i.e., high in organic content); however, the suitability of such substrates needs to be validated by ad-hoc, long-term experimental tests.

Based on the characterization of the wastewater streams and the most recent experimental work on abattoir wastewater and microalgae harvesting (Maroneze et al., 2014; Hernandez et al., 2016), two alternative scenarios are identified at Site G:

- Scenario A: The effluent from the anaerobic pond is used as substrate for microalgae growth system, thus bypassing the current aerobic treatment process and reducing the size of further storage downstream. The low concentration of FOG and BOD (8 ± 5 mg/L and 54 ± 54 mg/L for FOG and BOD, respectively) and high nutrient content (151 ± 42 mg/L and 26 ± 7 mg/L for TN and TP respectively) are within literature values and suitable for microalgae cultivation system.
- Scenario B: The primarily treated wastewater effluent is used as a substrate for microalgae cultivation, thus substituting the current biological treatment (i.e., anaerobic and aerobic) with microalgae. The concentration of FOG and COD (179 ± 219 mg/L and 1,328 ± 2,080 mg/L for FOG and COD, respectively) might limit the growth of microalgae on the primarily treated wastewater effluent, although literature studies suggest microalgae could grow well on similar substrates.

The characterization of the wastewater streams at Sites A, C and D has shown that a relatively low concentration of organics and high content of nutrients are found in streams generated from the wash down of cattle and kill floor. Combining these two wastewaters leads to a resulting stream (COD from 2,000 to 3,400 mg/L; FOG from 70 to 320 mg/L; TKN from 150 to 700 mg/L; TP from 18 to 25 mg/L) that could potentially be used as substrate for microalgae growth, in accordance with the experimental conditions tested in recent literature studies (Maroneze et al., 2014; Hernandez et al., 2016). However, the reconfiguration of the current flowsheets for the cattle wash down and kill floor streams to by-pass AD and go straight to microalgae cultivation would cause considerable changes in the current operations without a significant improvement of the final stream composition ahead of the microalgae process (refer to Appendix 9.3 for detailed calculations). For this reason, at Sites A and D, it is recommended to limit the application of the microalgae cultivation system on the AD effluent only. However, if a new wastewater treatment process has to be designed for an abattoir, by-passing AD and going straight to microalgae cultivation might be a cost-effective option for some wastewater streams generated within the abattoir's operations. In this configuration, blending of all streams prior microalgae as well as treating each stream in a dedicated microalgae cultivation system (i.e., with an algae culture specific for each wastewater streams) are two options that are worth assessing.

Similarly, given the current process flowsheet, the AD effluent generated at Site G provides the optimal solution for integration with the microalgae cultivation process. The low strength wastewater generated at Site G seems particularly suitable for a microalgae cultivation process as the concentration of organics and nutrients in the post AD stream is well within the range for microalgae growth.

Based on the assumptions on water quality and flowrate data and on the process flowsheet currently implemented at the reviewed abattoirs (refer to Appendices 9.1 and 9.3 for details), the AD effluent has been identified as the most suitable stream for integration with a microalgae cultivation system. The AD effluent compositions at Sites A, D and G are summarized in Table 9.

Table 9. Wastewater streams selected for integration with the microalgae cultivation system. NA: not available data. Italics highlighted values are estimated based on literature data and assumptions made by the authors.

	Site A	Site D	Site G
Wastewater stream	AD effluent	AD effluent	AD effluent
Volume (kL/d)	*2,423*	*2,150*	*1,750*
TCOD (mg/L)	700	1,100	54 ± 54 as BOD
TS (mg/L)	NA	NA	NA
FOG (mg/L)	*257*	*136*	8 ± 5
TKN (mg/L)	245	254	151 ± 42 as TN
NH_4-N (mg/L)	239	229	NA
TKP (mg/L)	38	34	26 ± 7 as TP
PO_4-P (mg/L)	33	32	NA

5.4 Milestone 4. Algae-cultivation system design

5.4.1 Microalgae cultivation approach

Algal cultivation systems have evolved in different technologies. The two most common cultivation system designs are known as open ponds and closed photobioreactors (Borowtizka and Moheimani, 2013); the first being commonly used for large-scale commercial production in favorable climatic conditions (Craggs et al., 1997; Raes et al., 2014). Although originally used only to hold wastes, open ponds were observed to reduce pollution such as organic matter and nutrient concentrations by allowing the growth of bacteria and microalgae (Benemann et al., 1977). Since then, they have been used as the main technology to harvest microalgae for treatment of municipal, industrial and agricultural wastewaters (Oswald, 2003). The most accepted open pond cultivation systems are paddlewheel driven raceway ponds which are currently utilized in large scale commercial applications due to their low capital expenditure and simple operation. Open raceway ponds are shallow ponds (i.e., normally between 15 to 25 cm deep), equipped with a paddle wheel mixing mechanism to keep the microalgae suspended in the water. They use atmospheric CO_2 (with some additional CO_2) to reach high biomass productivity. Biomass concentrations of up to 1 g/L dry weight and productivities of 60-100 mg/L/d dry weight have been reported in commercial applications

(Mohemiani, 2005). The algal biomass produced and harvested from these systems can be converted through various pathways to biofuels, for example recycling to AD for biogas production, transesterification of lipids to biodiesel, fermentation of carbohydrate to bioethanol and high temperature conversion to bio-crude oil (Park et al., 2011). A large footprint, potential contamination by unwanted algal species and other organisms, and challenging control of operating parameters (e.g., temperature, light) are the main disadvantages of open raceway ponds (Raes et al., 2014; Nwoba et al., 2016; Chaudry et al., 2017). Evaporation losses and a low microalgal biomass (e.g., 0.5 g/L) induced by poor mixing and low light penetration are also some potential limitations (Chaudry et al., 2017).

Closed photobioreactors (PBRs) are an alternative microalgae cultivation system. Closed PBRs are made up of transparent material which can pass light. The major operational difference between open ponds and closed PBRs is related to the number of biotic and abiotic factors that can be regulated to optimize and stimulate growth, thus leading to high productivity in closed PBRs. Other distinct advantages of PBRs over raceway ponds are the absence of evaporation and reduction of contamination by unwanted species. Big bag system, flat plate (vertical or inclined) and tubular (serpentine type or Biocoil) reactors are different types of closed PBRs. The main disadvantage of the use of closed PBR is the very high capital and operational costs (Moheimani, 2005) which makes its selection less favorable for commercial applications and has limited their applicability on a large scale. Amongst typical limitation of the process itself, closed PBRs require a cooling system, which increases operational costs, and have shown a greater oxygen build-up, which reduces productivity (Raes et al., 2014).

Both open ponds and closed PBRs have been tested at experimental scale in microalgae cultivation experiments on pre and post AD abattoirs wastewaters (Hernandez et al., 2016; Taskan, 2016). Although biomass productivity is normally higher in PBRs, similar ammonium removal rate has been found in open ponds and PBRs (Nwoba et al., 2016). This result could be a consequence of the fact that removal of ammonia in open ponds is not only biological (e.g., uptake by microalgae) but also due to stripping in open atmosphere. Due to their commercial maturity, simplicity of operations, longer durability, lower capital and operational costs comparing to PBRs (Mohemiani, 2005), paddlewheel driven open raceway ponds are the technology of choice for the cultivation of microalgae in this study.

Several microalgae have been reported as good candidates for wastewater bioremediation including *Chlamydomonas* sp., *Euglena* sp., *Micractinium* sp., *Botryococcus* sp., *Coelastrum* sp., *Chlorella* sp., *Scenedesmus* sp., *Oscillatoria* sp. and *Spirulina* sp. (Nwoba et al., 2016). It is now common practice to favor the cultivation of a microalgae consortium over monocultures because of simplicity of operations and also because single microalgal strains find it difficult to remove all the nutrients simultaneously from wastewaters. *Chlorella* and *Scenedesmus* have shown to be highly robust and versatile due to their tolerance to different wastewater conditions. Ayre et al. (2017) and Nwoba et al. (2016) reported *Chlorella* sp., *Scenedesmus* sp. and a pennate diatom can grow efficiently on undiluted AD piggery effluents with up to 1,600 mg/L NH_4-N, although previous investigations have found high ammonia concentrations toxic to microalgae (high NH_4^+ concentrations at pH higher than 8 shift the chemical equilibrium towards NH_3 which is considered toxic for algae). Similar results on the growth of *Chlorella* sp. on undiluted piggery slurry have been found by Wang et al. (2016).

In our study, the choice of which microalgal species to harvest will be determined by the suitability of the identified strain to grow on abattoir wastewaters and by the final use of the cultivated algal biomass. *Chlorella* and *Scenedesmus* sp. are found suitable as a source of animal feed or biogas by direct recycle to the AD lagoon (Nwoba et al., 2016). Species of *Botryococcus* have shown a high oil content (up to 75% by weight), which can be extracted and then converted/upgraded to high quality liquid biofuel (Chaudry et al., 2017). Due to its well-known dynamics, *Chlorella* sp. is used in this project as the microalgae of reference. Modelling assumptions concerning algae productivity, methane production and market value, are based on literature and experimental values of *Chlorella* growth.

5.4.2 Mass and energy balances at selected abattoirs

The results of the mass and energy balances calculated at Sites D and G are presented below. Note that all the models consider the AD effluent as the optimal substrate for microalgae cultivation (Table 9).

Site D – Mass and energy balances

The results of the mass balance of the microalgae cultivation system on the AD effluent at Site D are shown in Figure 5. For the calculation of the carbon dioxide available for algae growth, the methane production potential of the pre AD stream has been considered equal to 9,783 m^3/d (Jensen and Batstone, 2013). A Redfield ratio C:N:P = 196.5:15.8:1 is calculated, which makes nitrogen the nutrient limiting the growth of microalgae. Based on the stoichiometric reaction (Eq. 1) and the concentration of nutrients in the AD effluent, a theoretical algae biomass production of 5,221 kg/d is calculated (Figure 5). As nitrogen is the limiting nutrient, its percentage of removal from the water stream is 100%. About 99% of the incoming phosphorous is expected to be consumed daily by algae growth.

Recent studies conducted at Murdoch University have demonstrated the suitability of *Chlorella* sp. to grow on wastewaters characterized by very high ammonia and turbidity, thus further demonstrating the ability of this microalga to grow on challenging substrates. Borowitzka (1992), Lundquist et al. (2010) and Collet et al. (2011) consider an annual average productivity of *Chlorella* equal to 25 g/m^2/d (Table 1). At a pond depth of 30 cm (Table 1), a total area of open raceway pond equal to 21 ha is needed to grow the estimated 5,221 kg of algae biomass per day (Figure 5). A significant amount of water, equal to 856 kL, is expected to evaporate daily, thus an equivalent volume of water is needed to be added to maintain the concentration ratio of nutrients and biomass.

At full scale operations, the microalgae cultivation system is expected to operate at steady state, thus the harvesting rate equates the rate at which algae biomass grows (i.e., 5,221 kg/d). Assuming a concentration of algae in the ponds equal to 0.5 g/L (Collet et al., 2011) and an efficiency of harvesting and dewatering equal to 90% (Collet et al., 2011), a flow rate of 11,603 kL is harvested daily from the ponds (Figure 5). After cultivation and harvesting, dewatering of algae is required to remove water from the algae biomass. In current commercial microalgae production plants, harvesting and dewatering represents a significant part of the overall production costs (Chaudry et al., 2017). Microalgae dewatering methods can be performed in two stages. Primary dewatering (thickening) is done by settling and flocculation, achieving a 20-fold concentration factor and a 1% solids biomass. Secondary dewatering includes further filtration and centrifugation and allows achieving concentration of biomass up to 30% solids. At this concentration, the microalgae product can be used for a variety of applications, such as extraction of oil and pharmaceutical products, on-site animal feed and fertilizer. Further dewatering by heating and drying allows reaching as high as 90% solids concentration of biomass, which is required whether transportation of the algae biomass products for off-site uses is necessary. The obtained dry biomass can be used very efficiently for many applications, such as recycle to animal feed, oil extraction and biofuel production, as well as being transported at lower costs. At a 90% efficiency of the dewatering process and a final 30% solids biomass stream, the algae biomass grown at Site D and output of the cultivation and dewatering systems achieves a concentration of 300 g/L and a mass of 5,221 kg/d at a flow rate of 17 kL/d (Figure 5).

Together with a dewatered microalgae biomass product, the other output of the system is a stream of water that is depleted in nutrients (Figure 5). Part of the clean water product is used within the microalgae process to make up for evaporation losses (Figure 5), and the remaining clean water (1,276 kL/d, Figure 5) can be recycled back to the abattoir or discharged. Further filtration and treatment to remove the remaining algae biomass in the nutrient-depleted water stream might be necessary before recycle.

The daily energy demand of each system's process is highlighted in Figure 5 and summarized in Table 10. Unit energy consumption rates refer to literature values summarized in Table 1. A daily and annual energy demand of 3.5 MWh and 1.2 GWh, respectively, has been estimated for the treatment of the AD effluent by microalgae cultivation at Site D (Table 10). For a 21 ha system, the energy consumption relative to pond mixing by a paddlewheel mechanism is equal to 28% of the overall energy consumption and 16 MWh/ha/y, which sits at the lower end of the 19 to 28 MWh/ha/y range given for open pond cultivation in Australia (Campbell et al., 2011). The dewatering system achieving a 30% solids output by centrifugation represents 41% of the total daily energy consumption (Table 10).

Table 10. Energy consumption of the microalgae cultivation system at Site D. Wastewater stream sent to microalgae cultivation: AD effluent.

Energy demand	kWh/day	% of total
Paddlewheel mixing	1,002	28.3
Harvesting and dewatering	1,450	40.9
Water pumping	687	19.4
CO_2 pumping	403	11.4
Total energy demand	3,543	100

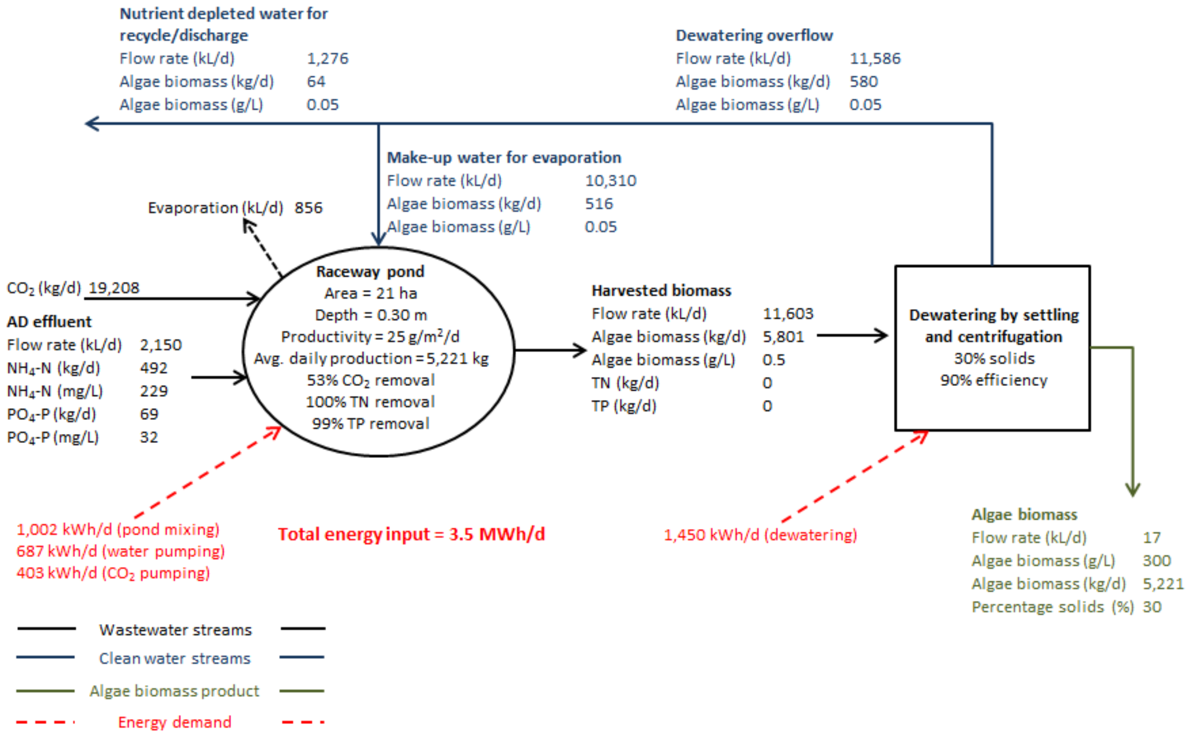


Figure 5. Mass balance and system sizing of the microalgae cultivation system at Site D. Wastewater stream sent to microalgae cultivation: AD effluent.

Site G – Mass and energy balances

The results of the mass and energy balance calculations of the microalgae cultivation system on the post AD effluent at Site G are summarized in Figure 6 and Table 11. The introduction of a microalgae cultivation step on the AD effluent implies the substitution of the current aerobic treatment with the microalgae process (Appendix 9.3, Figure A3.1).

A theoretical algae biomass production of 2,802 kg/d is calculated at Site G (Figure 6). Nitrogen is found to be the nutrient limiting growth (C:N:P = 239.17:12.84:1), thus the resulting water stream is expected to be 100% depleted in nitrogen. The efficiency of removal of phosphorous is calculated at 80% and a final concentration of TP in the water is estimated at 1.4 mg/L. Note that the nitrogen and phosphorous concentrations in the AD effluent are measured by Site G personnel as total nitrogen and phosphorous, and measurements of ammonia and phosphate are not available. Although it is realistic to assume the majority of post AD nitrogen and phosphorous is in the form of ammonia and phosphate, this assumption will need to be validated with a more detailed characterization of the post AD stream. At Site G, the methane generated in the anaerobic pond is not currently being captured. Communication with Site G personnel advised that capturing the methane is amongst the abattoir's short-term priorities. In the mass balance calculations, it is assumed that a methane production potential of 4.5 m^3 CH_4 per kL of wastewater is produced by anaerobically digested slaughterhouse wastewaters (Jensen and Batstone, 2012 and 2013), thus producing 7,875 m^3 CH_4/d. Considering combustion of one mole of methane produces one mole of CO_2 (Eq. 2), enough carbon dioxide will be generated daily for the estimated algae biomass of 2,802 kg/d.

At an algae productivity value of 25 g/m^2/d and a pond depth of 30 cm, a total area of open raceway pond equal to 11 ha is needed to grow the estimated mass of algae per day. Considering the same harvesting and dewatering system used for Site D is also applicable on Site G, the final microalgae product is a stream with a concentration of biomass equal to 300 g/L and a daily flow rate of 9 kL (Figure 6). The resulting nutrient-depleted clean water stream has a total volume of 1,281 kL/d (Figure 6).

A daily and annual energy demand of about 1.8 MWh and 604 MWh, respectively, has been estimated for the treatment of the AD effluent by microalgae cultivation at Site G (Table 11 and Figure 6).

Table 11. Energy consumption of the microalgae cultivation system at Site G. Wastewater stream sent to microalgae cultivation: AD effluent.

Energy demand	kWh/day	% of total
Paddlewheel mixing	538	29.4
Harvesting and dewatering	778	42.5
Water pumping	398	21.8
CO_2 pumping	116	6.3
Total energy demand	1,830	100

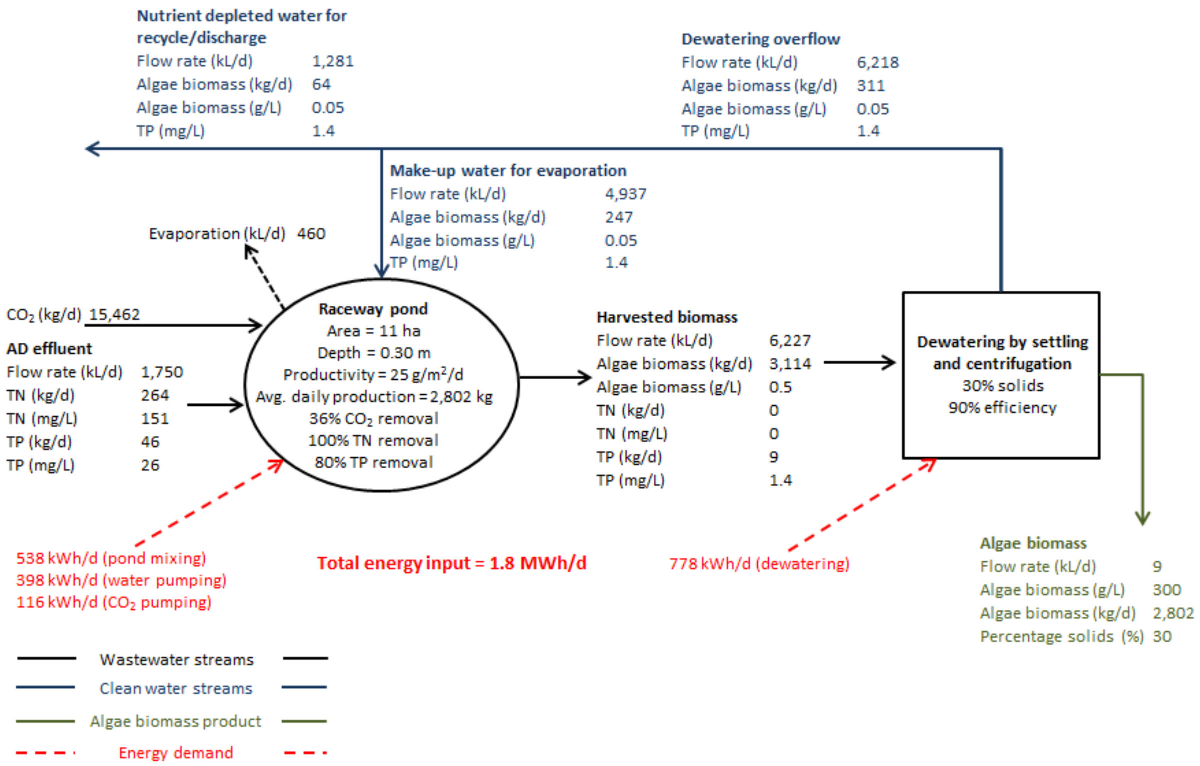


Figure 6. Mass balance and system sizing of the microalgae cultivation system at Site G. Wastewater stream sent to microalgae cultivation: AD effluent.

5.4.3 Mass and energy balances of the anaerobic digestion of algae biomass

The variety of uses associated with algae biomass makes it a very versatile product for abattoirs as it can be used to both produce additional revenue by selling algae products (e.g., animal feed and fertilizer) and/or offset some of the environmental impacts generated from the wastewater treatment, such as its energy demand and GHG emissions. Site D abattoir has been used as the reference site to integrate the mass and energy balances developed in Figure 5 with an AD step on the cultivated algae biomass. The objective is to assess the feasibility of producing biogas, i.e., electricity, from algae whilst minimizing the re-dissolution of nutrients in the final clean water product. The methodology developed by Collet et al. (2011) and the experimental results on AD of *Chlorella vulgaris* found by Ras et al. (2011) are used to model the process at Site D. The results of this modelling exercise quantify the energy demand (as the identified most important environmental impact) of the integrated AD-microalgae cultivation system where the whole, or a fraction of, algae biomass is used to produce electricity as methane biogas.

As discussed earlier, the products of microalgae cultivation are a nutrient-depleted effluent that can be discharged and/or recycled, and an algae biomass stream concentrated at 30% solids. The main environmental impact associated with this process is the energy demand of 3.5 MWh/day (also equal to 2.5 MJ/kg of cultivated algae), required mainly for pond mixing and algae dewatering (Table 10). The estimated energy demand is at the high range of the LCA studies reviewed by Handler et al. (2012), who determined an energy requirement associated with the cultivation and dewatering stages ranging between 0.6 to 2 MJ/kg of algae. Our estimate at Site D is therefore quite conservative and a lower energy demand might be found in the practice.

Amongst the identified possible uses of the cultivated biomass, its use as animal feed and fertilizer are potentially the most straightforward and beneficial for the abattoir. However, whether the biomass is to be sold and transported off-site, further drying of the biomass to a 90% solids product is required, thus increasing the energy demand of the overall process. The opportunity of using the whole, or a fraction of, algae biomass through AD to produce electricity and offset 3.5 MWh/day energy demand is also an attractive option to improve the environmental footprint of the abattoir. The process flowsheet diagram of the integrated AD-microalgae scenario is shown in Figure 7. Note that dewatering of algae biomass before AD only requires a 5% solids product output of centrifugation, thus energy savings of the cultivation-dewatering process are expected in the integrated AD-microalgae process. The degradation of the microalgae biomass during AD is a mature process that is expected to produce enough electricity to offset the 3.5 MWh/day energy demand. However, at an HRT long enough to promote maximum methane production, a nearly complete mineralization of nitrogen and phosphorous occurs, thus causing the release of nutrients in the liquid digestate (i.e., liquid stream output from AD, Figure 7). Even if the liquid digestate is blended with the nutrient-depleted water recycled from the algae dewatering step, the concentration of nutrients might reach too high values for recycling and disposal. Nutrient mineralization by AD can be controlled by keeping the HRT of the AD process short; however, short HRTs would generate less methane, thus less electricity. In order to analyze the tradeoff between methane production and nutrient mineralization at Site D, some scenarios of the integrated AD-microalgae cultivation process are modelled hereafter. The amount of generated electricity and the nutrient concentration in the blended water (liquid digestate plus nutrient-depleted water, Figure 7) are quantified as a function of the fraction of algae biomass sent to AD and the HRT of the AD process. The performances of the AD of the microalgae *Chlorella* at HRTs of 16, 28 and 46 days are derived from Collet et al. (2011) and Ras et al. (2011) and summarized in Table 12. The results of the different modelled scenarios are presented in Table 13.

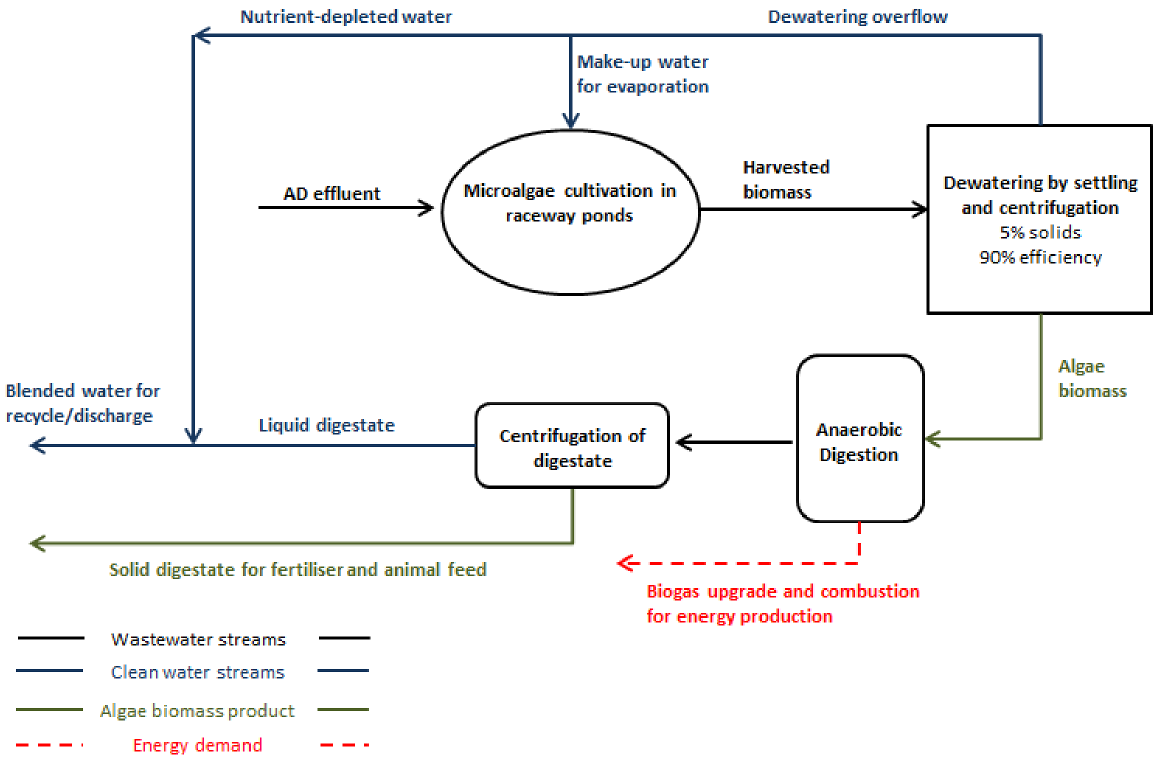


Figure 7. Schematic process flowsheet diagram of the integrated AD-microalgae cultivation system.

Table 12. Anaerobic digestion performances of microalgae *Chlorella* at different hydraulic retention times (HRT) of the AD process. Experimental data derived from Collet et al. (2011) and Ras et al. (2011).

Parameters	Value		
HRT of anaerobic digestion (days)	16	28	46
Methane fraction in biogas (%)	30	50	70
Methane conversion (mLCH_4/gVSS)	147	240	292
Nutrient mineralization (%)	19	68	90
COD removal (biodegradability of carbon fraction) (%)	33	51	56
VSS fraction (gVSS/gTSS)	0.9	0.9	0.9
COD fraction (gCOD/gVSS)	1.37	1.37	1.37
Lower heating value of biogas (kWh/m^3)	3[a]	6.11[a]	6.11[a]

[a] Value of 6.11 kWh/m^3 (22 MJ/m^3) derived by Banks presentation (online at: http://www.valorgas.soton.ac.uk/Pub_docs/JyU%20SS%202011/CB%204.pdf). The value of 3 kWh/m^3 at 30% CH_4 biogas is estimated by the authors.

Table 13. Results of modelled anaerobic digestion of algae biomass produced daily at Site D as a function of the HRT and the biomass fraction sent to AD.

	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
AD operating conditions						
HRT (days)	46	46	46	28	28	16
Fraction of biomass to AD (%)	100	15	50	100	50	100
AD Input						
Flow rate (kL/d)	104	16	52	104	52	104
Algae biomass (kg/d)	5,221	783	2,611	5,221	2,611	5,221
Algae biomass (g/L)	50	50	50	50	50	50
AD Output						
Biogas (m^3/d) [a]	1,379	207	689	1,587	793	937
Energy generation (kWh/d) [b]	9,994	1,499	4,997	8,215	4,107	2,911
Energy demand (kWh/d)	3,415	2,472	2,860	3,478	2,892	3,282
Energy offset (%) [c]	293	61	175	236	142	89
N in blended water (mg/L) [d]	344	55	179	260	135	73
P in blended water (mg/L) [d]	47	8	25	36	19	10

[a] Net amount of biogas after internal recycle to AD for heat supply

[b] Based on the conversion of biogas to electricity as given in Collet et al. (2011) and equal to 9.94 kWh/m^3 of biogas

[c] Percentage calculated as the energy produced by AD of algae biomass in each scenario divided by total energy demand

[d] Concentration of nutrients in the final blended water for recycle/discharge resulting from blending liquid digestate with nutrient-depleted water

In order to offset the energy demand of the integrated AD-microalgae cultivation system and produce excess electricity, more than half of the entire biomass needs to be anaerobically digested at an HRT long enough to promote full methanisation (longer than 28 days, Scenario 1, 3, 4 and 5, Table 13). This process, however, causes the re-dissolution of 60 to 80% of the nutrients in the liquid digestate, leading to their concentration in the blended water equal, or even higher, than those measured in the abattoir secondarily treated wastewater. In order to limit the mineralization of nutrients to levels that would allow the final blended water to be recycled or discharged (about 40 mg/L for N and 12 mg/L for P, Table A4.1), either a small fraction of biomass at long HRT (15% only, Scenario 2, Table 13) or the whole biomass at a short HRT (16 days, Scenario 6, Table 13) are to be treated through AD. In both cases the amount of produced electricity is enough to offset more than half of the AD-microalgae system energy demand (61% and 89% for Scenario 2 and 6, respectively, Table 13), whilst keeping the concentration of nutrients in the blended effluent within acceptable levels (about 55-70 mg/L for N and 8-10 mg/L for P, Table 13).

Amongst the modelled scenarios, Scenario 6 seems to be the most appropriate. The short HRT limits the re-dissolution of nutrients to a concentration in the final blended water that is adequate for recycling, whilst generating about 90% of the energy demand of the AD-microalgae cultivation system. The solid digestate and remaining post AD algae biomass is still high in protein content, thus making it a suitable source of animal feed. The mass and energy balances of the integrated AD-microalgae process modelled in Scenario 6 are summarized in Figure 8 for Site D and in Figure 9 for Site G.

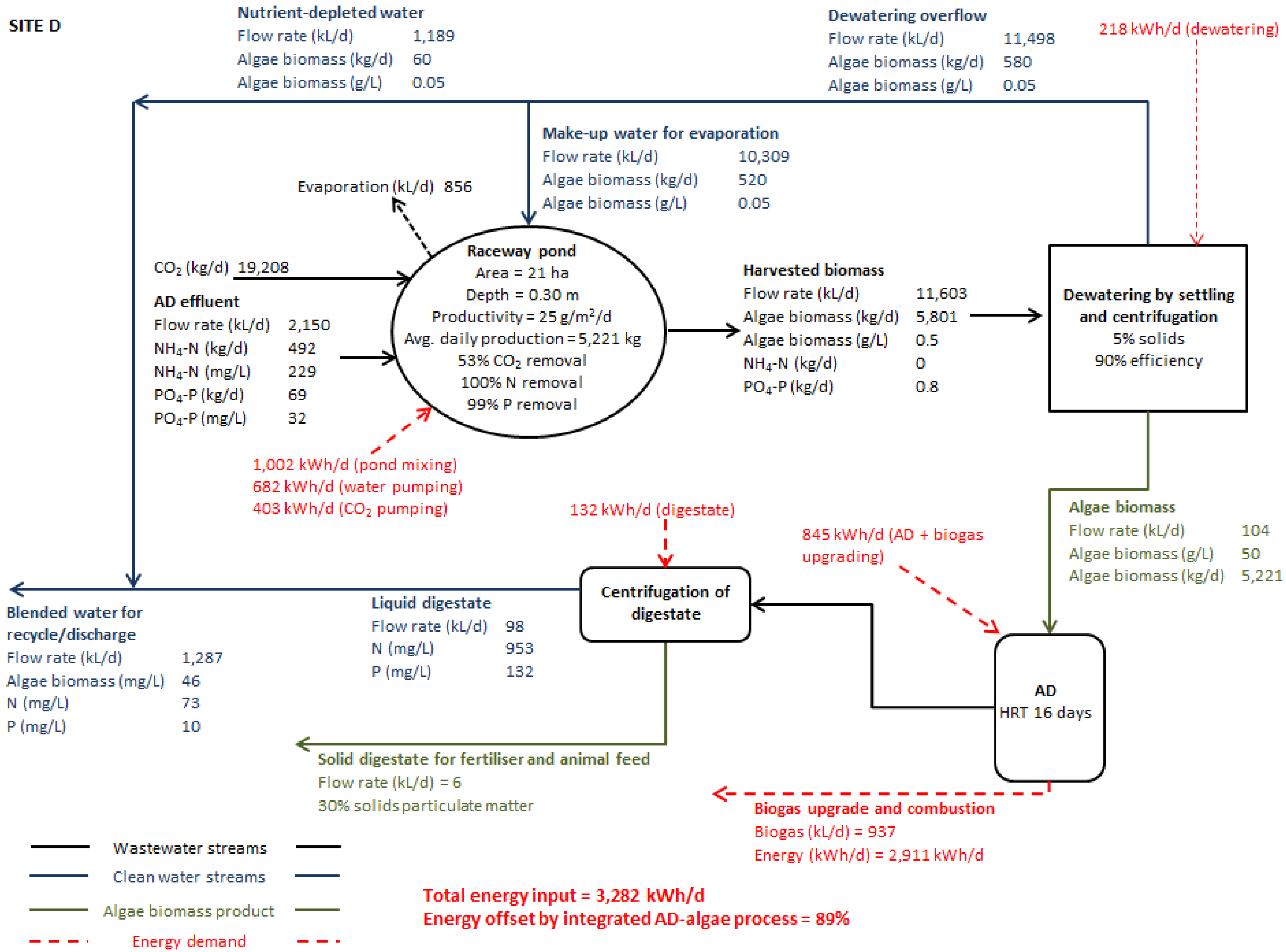


Figure 8. Mass and energy balances of the integrated AD - microalgae cultivation system at Site D.

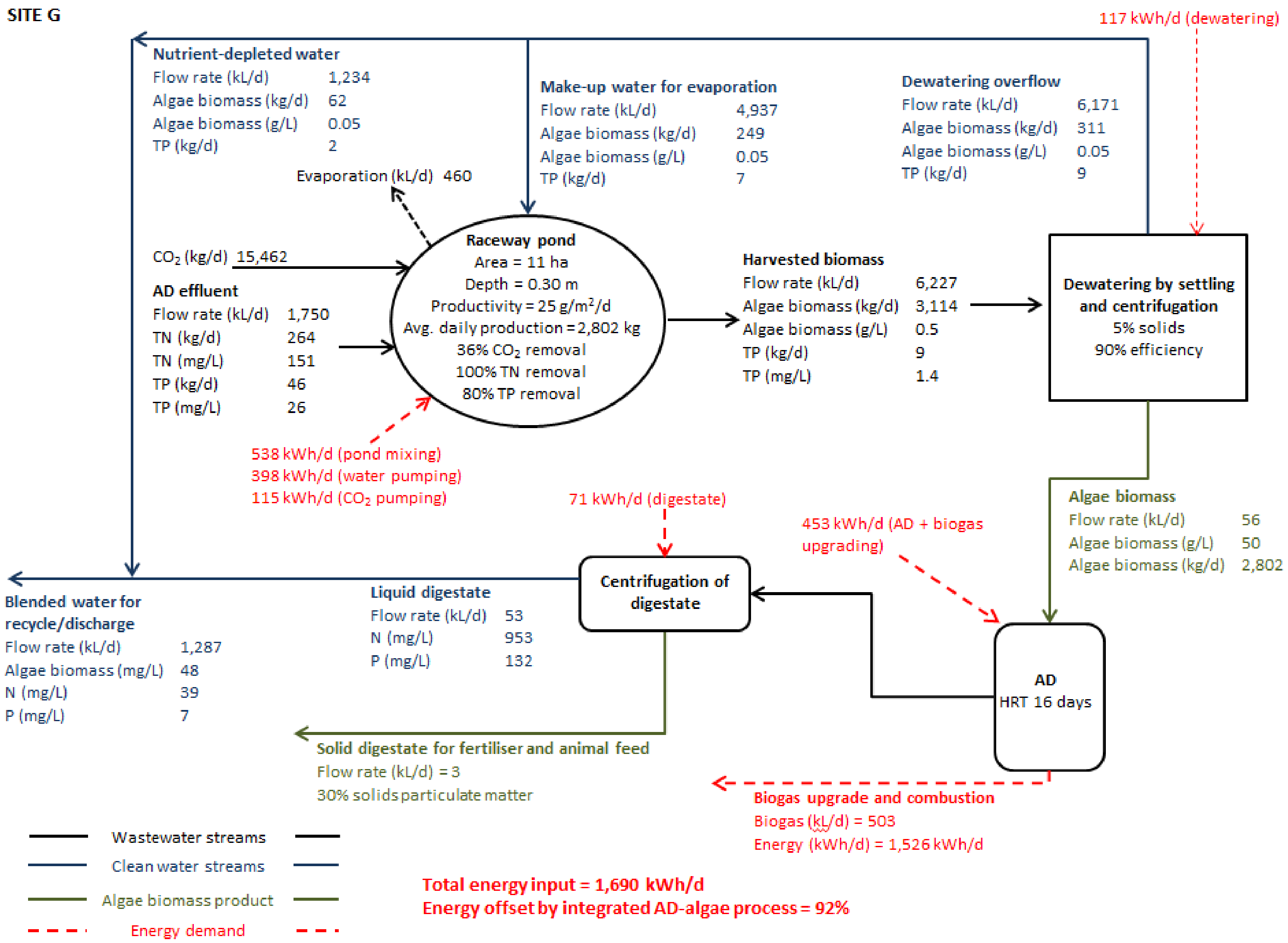


Figure 9. Mass and energy balances of the integrated AD - microalgae cultivation system at Site G.

5.5 Milestone 5. Environmental impact analysis

The full environmental impact analysis developed in Milestone 5 is reported in Appendix 9.4. A summary of the main results is given below.

The results of the qualitative environmental impact and risk assessment identify several positive environmental impacts of the proposed microalgae cultivation system, as opposed to the negative environmental footprint of current practices. The conventional treatment and management of AD wastewater effluents currently implemented in abattoirs most often include the use of a small fraction of the treated effluent for irrigation, with the majority of it stored indefinitely in evaporation ponds. Such practice leads to mostly negative environmental impacts related to:

- Generation of large volumes of water with high nutrient concentrations, thus not suitable for on-site recycle, except for irrigation;
- Possible greenhouse gas emissions due to storage in open ponds of large volumes of water with elevated content of ammonia nitrogen;
- Potential eutrophication of soils and surface waters, nutrient imbalance, pest and disease in plants due to the high content of nitrogen and phosphorous in the water used for irrigation;
- Potential nitrogen contamination to groundwater;
- Inefficient use of large areas of land dedicated to evaporation and storage ponds;
- Loss of the environmental and economic value of the treated wastewater effluent associated with potential resource recovery of water, nitrogen and phosphorus.

Further treatment to reduce nutrient concentrations by appropriate technologies is the main preventative measure to minimize the identified environmental impacts and risks.

Positive and negative environmental impacts have been identified when a microalgae cultivation process is integrated in the treatment flowsheet. The main negative environmental impact is related to the on-site recycle of the wastewater effluent and is identified as the potential contamination of the potable water used for meat processing operations. Preventative measures to reduce this risk to acceptable levels are required, such as different reticulation systems for potable and recycled water, proper personnel training and limited permitted on-site uses of recycled water. Another negative environmental impact relates to the large land footprint dedicated to microalgae cultivation in raceway ponds; however, this is also a drawback of open ponds storage and other suggested cost-effective technologies for nutrient removal (e.g., wetlands). Several positive environmental impacts of growing microalgae on the AD effluents have been identified:

- Reclamation of the environmental and economic value of the wastewater effluent in terms of water and nutrient recovery;
- Generation of large volumes of water that are suitable for recycling on-site and off-sit or safe to discharge to surface water bodies;
- Generation of an algae biomass product that is suitable for re-use with the abattoir's operations or for sale to available markets;
- Sequestration of carbon dioxide currently generated by the conventional wastewater treatment

process (e.g., AD) and fixation into algae biomass.

5.6 Milestone 6. Cost benefit and uncertainty analysis

5.6.1 Cost benefit analysis of the proposed microalgae cultivation system

The estimate of capital and operational costs of the microalgae cultivation system is summarized in Table 14 for Site D and Site G, for both scenarios with and without AD. Total CAPEX and annual OPEX are reported separately and the contribution of each cost element is shown in Figure 10. Note that the same cost estimates per hectare have been used for Site G and D (refer to Table 2, Section 4.5), therefore the contribution of each cost element shown in Figure 10 is the same for Site G and D. As an indicative estimate, the annual expenditures of CAPEX and OPEX are calculated for the case where all of the capital cost is sourced from a bank loan. In this case a 10-year lifetime project is assumed with an annual interest of 6.35% (Wijihastuti, 2017). Costs are also given per unit area and per unit of cultivated algae biomass (Table 14). Estimates below are all expressed in Australian dollars.

A total CAPEX of M$ 3.9 and 2 are calculated for Site D and G respectively (Table 14), half of which is due to the construction of raceway ponds and the necessary infrastructures (buildings, roads, water pumping and electrical system, Figure 10a). OPEX costs amount to M$ 2.1 and 1.3 per year (Table 14). The largest contribution to OPEX is represented by the costs associated with maintenance, insurance and taxes on the capital infrastructures (Figure 10b). Decreasing initial capital is therefore going to significantly decrease the annual operational costs as well. It should be noted that the costs associated with labour (about 20% of the total OPEX, Figure 10b) are likely to be overestimated as they refer to a newly built and stand-alone wastewater treatment plant. In the case of existing wastewater treatment plants operating on the abattoir premises, the additional labour costs associated with the microalgae cultivation system could be partially absorbed by the existing personnel, thus potentially reducing the labour costs reported in Table 14. Finally, the electricity required to mix the ponds, pump water and dewater the algae biomass up to a 30% solids product, represents a small fraction of the total OPEX (Figure 10b).

In the case of the integrated AD-microalgae cultivation system, CAPEX costs increase from M$ 3.9 and 2 for Site D and G to M$ 5.6 and 3 for Site D and G, respectively (Table 14). The higher CAPEX is due to the added capital associated with anaerobic digesters, gas turbines to burn methane and digestate centrifugation (Figure 10c). Although the electricity costs are almost reduced to zero (Figure 10d) and the labour costs are equivalent to the scenario without AD, OPEX costs are higher due to the maintenance, insurance and tax related costs associated with a higher CAPEX. The total annual expenditures and the unit production costs of algae are also higher in the case where AD on the grown biomass is integrated to the process (Table 14 and Figure 11).

Given its higher CAPEX, OPEX and unit costs of production, the integrated AD-microalgae cultivation system does not seem to represent the best option for Sites G and D. Moreover, careful operations of the AD of the algae biomass to ensure minimal nutrient re-dissolution in the blended water (Figure 8 and 9) make the process increasingly complex. On the contrary, the simplicity and lower costs associated with the use of the algae biomass product as on-site fertiliser and animal feed make the scenario without AD an attractive option for abattoir operations. Moreover, the nutrient-depleted water can be directly recycled on-site or safely discharged to surface water bodies.

Table 14. Summary of CAPEX and OPEX estimate for Site D and G abattoirs.

		Microalgae cultivation system		Integrated AD - Microalgae cultivation system	
	Unit	Site D	Site G	Site D	Site G
Capital Costs					
Open raceway pond – clay lined	AU$	1,036,299	556,194	1,036,299	556,194
Buildings, roads, drainage, vehicles	AU$	140,098	75,192	140,098	75,192
Electrical	AU$	577,410	309,903	577,410	309,903
Water piping	AU$	425,460	228,349	425,460	228,349
CO_2 (flue gas+ distribution)	AU$	180,517	96,885	180,517	96,885
Dewatering system	AU$	368,631	197,848	368,631	197,848
Digesters	AU$	0	0	665,541	357,204
Biogas turbine	AU$	0	0	741,516	397,980
Inoculum system	AU$	334,081	179,305	334,081	179,305
Engineering fees	AU$	459,374	246,551	670,433	359,829
Contingency	AU$	153,125	82,184	223,478	119,943
Working capital	AU$	183,750	98,621	268,173	143,932
Total CAPEX	AU$	3,858,742	2,071,032	5,631,633	3,022,563
Operational costs					
Electricity	AU$/year	315,675	163,085	33,081	11,413
Labour - Plant Manager	AU$/year	113,520	113,520	113,520	113,520
Labour - Engineer	AU$/year	84,480	84,480	84,480	84,480
Labour - Lab Analyst	AU$/year	63,360	63,360	63,360	63,360
Labour - Administration	AU$/year	60,720	60,720	60,720	60,720
Labour - Technician/Pond Operator	AU$/year	100,320	50,160	100,320	50,160
Maintenance/ Insurance	AU$/year	385,874	207,103	563,163	302,256
Tax	AU$/year	1,010,623	542,413	1,474,952	791,624
Total OPEX	AU$/year	2,134,572	1,284,841	2,493,596	1,477,534
Annual instalment for a 10 years loan at 1.3% inflation rate and 6.35% bank interest					
Annual CAPEX	AU$/year	533,008	286,071	777,897	417,506
Annual OPEX	AU$/year	2,134,572	1,284,841	2,493,596	1,477,534
Total annual expenditures	AU$/year	2,667,580	1,570,913	3,271,493	1,895,040
Costs per unit area					
Pond area	ha	21	11	21	11
Total CAPEX	AU$/ha	184,763	184,763	269,652	269,652
Total OPEX	AU$/year/ha	102,207	114,625	119,398	131,815
Total annual expenditures	AU$/year/ha	127,728	140,146	156,645	169,062
Costs per unit production of algae biomass					
Total annual biomass	ton/year	1,723	925	1,723	925
Unit cost production of algae	AU$/kg	1.55	1.70	1.90	2.05

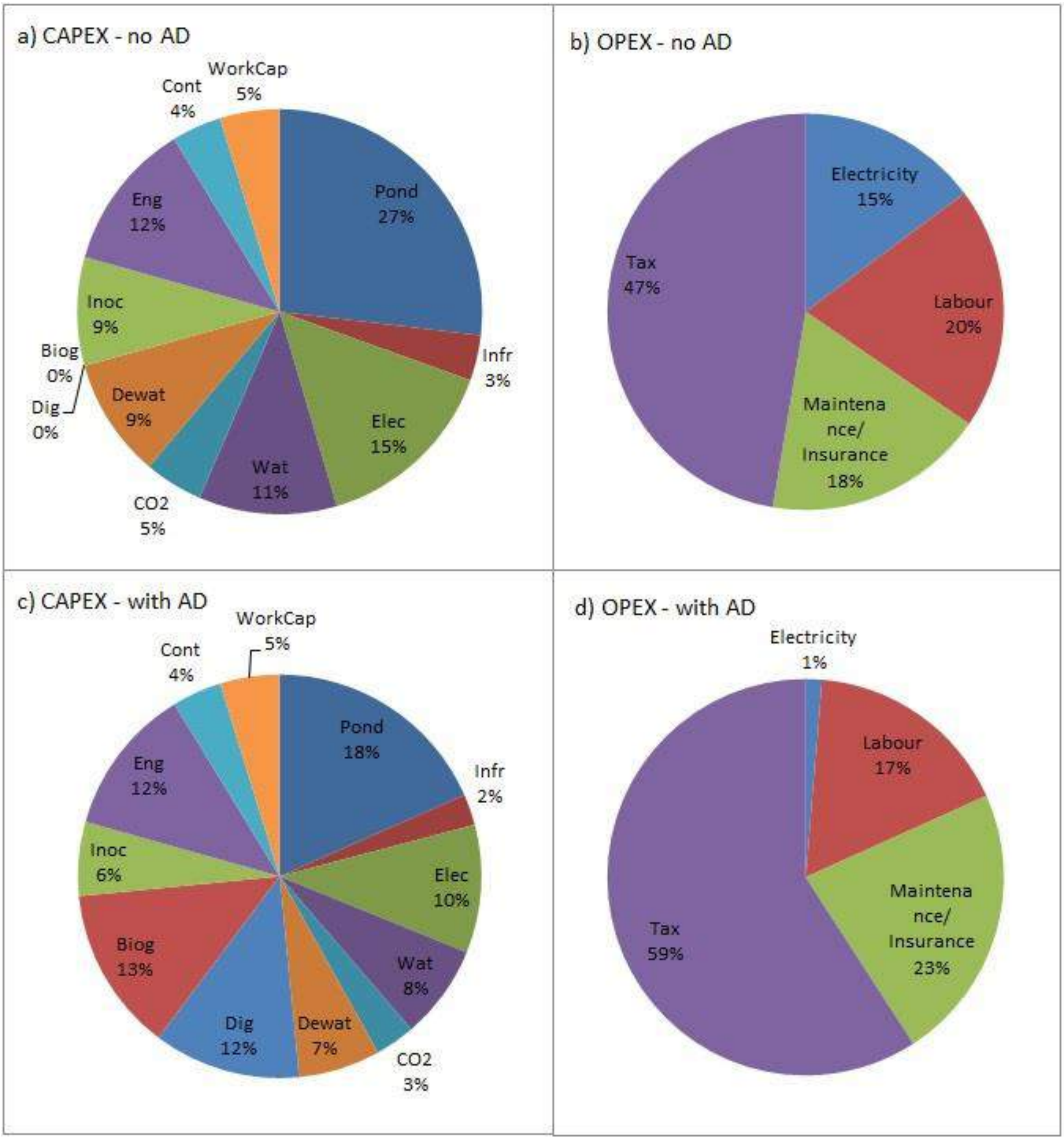


Figure 10. The contribution of different cost elements to the total CAPEX (a, c) and OPEX (b, d) for the microalgae cultivation system without (a, b) and with (c, d) anaerobic digestion. Capital cost elements: Pond = raceway pond; Infr = buildings, roads, etc; Elec = electrical layout; Wat = water piping; CO_2 = flue gas distribution system; Dewat = dewatering system; Dig = digesters; Biog = biogas trubines; Inoc = inoculum; Eng = engineering fees; Cont = contingency fees; WorkCap = working capital.

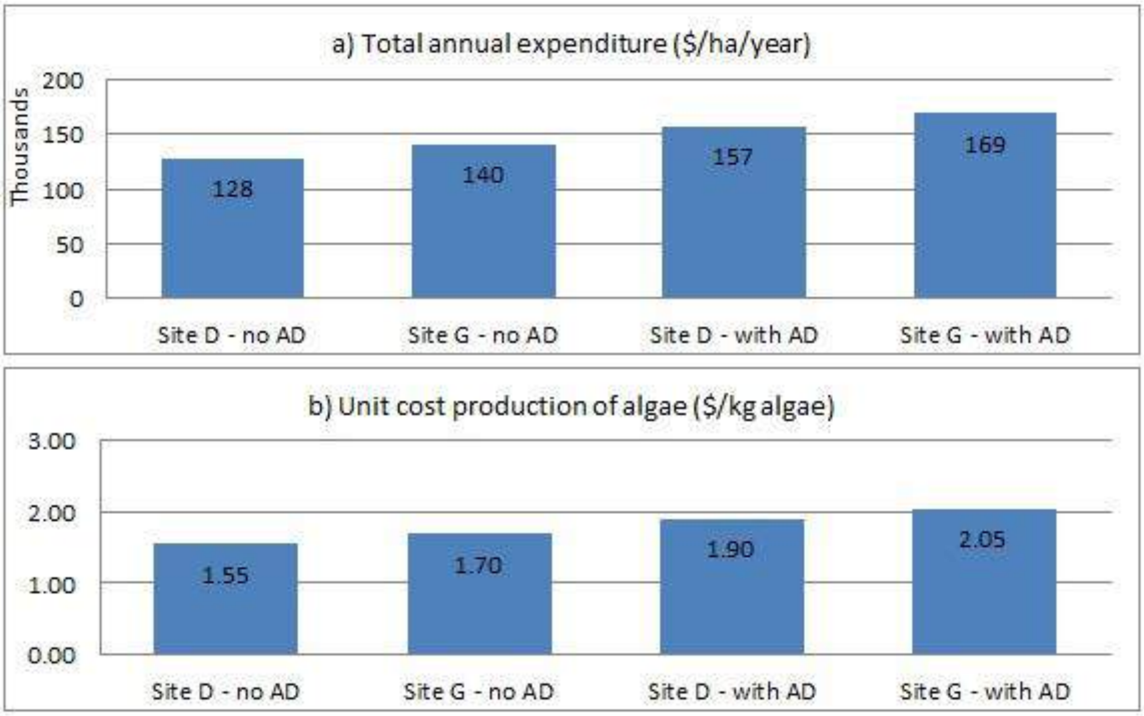


Figure 11. a) Total annual expenditures and b) unit production costs for Sites D and G.

5.6.2 Sensitivity and uncertainty analysis

The results of the mass and energy balances and the cost-benefit analysis have demonstrated the microalgae cultivation system without AD of the grown biomass as the most suitable, simple to operate and cost-effective option for the reviewed abattoirs. A sensitivity analysis of the mathematical model and economic analysis developed on Site G and D is performed to evaluate process uncertainties and modelling assumptions. The mathematical model and economic analysis as reported in Figure 5 and 6 and Table 14 constitute the base case of the following sensitivity analysis. The modelling parameters tested by the sensitivity analysis are summarized in Table 3 of the Methodology Section.

The sensitivity of process variables *i)* daily algae production (kg/d), *ii)* pond area (ha), *iii)* nutrient-depleted water for recycle (kL/d), *iv)* total CAPEX ($), *v)* total OPEX ($/year), and *vi)* algae production cost ($/kg) at each varying modelling assumption is represented as a percentage variation from the base case. As an example, the percentage variation from its base case value of the total CAPEX due to a +25% variation of algae productivity is calculated by Eq. 6. A positive percentage variation indicates an increase in CAPEX costs due to the +25% increase in algae productivity. Likewise, a negative percentage variation indicates capital costs decrease with increasing productivity. It should be noted that when one modelling parameter is varied, the others are kept constant and equal to their base case value. For example, when sensitivity analysis is performed on algae productivity, all the other parameters (e.g., nitrogen content in the wastewater, flow rate, pond construction costs, etc.) are equal to their base case values. Note that the energy costs associated with pond mixing and dewatering have only been varied between -25% to +25% from the base case as the cost analysis has shown a relatively low contribution of power costs to the overall capital and operational costs. A very low impact of pond mixing and dewatering energy consumption on the process variables have been found, thus results associated with these modelling parameters are not discussed further.

$$\frac{CAPEX_{+25\%\ productivity} - CAPEX_{base\ case\ productivity}}{CAPEX_{base\ case\ productivity}} = CAPEX\ \ Percentage\ \ Variation\ (\%) \quad \text{Eq. 6}$$

The results of the sensitivity analysis on Site D are summarized in Figure 12. Out of the nine modelling assumptions tested in the sensitivity analysis, the daily algae productivity per unit pond area (expressed as mass of algae grown per m^2 per day) is the most influential parameter. Although it doesn't influence the mass of algae grown daily (which is calculated based on stoichiometric and Redfield Ratio, Section 2, Eq. 1), the algae productivity significantly impacts on pond size, which in turn impacts on the amount of nutrient-depleted water effluent (through evaporation) and on capital, operational and unit production costs (Figure 12d). A decrease of algae productivity by 50% (equal to 13 $g/m^2/d$) causes a 90% increase in cultivation area and capital costs, as well as a 64% increase in OPEX and 70% increase in unit production costs. Augmented algae productivity by 50% (equal to 38 $g/m^2/d$) causes a 20% decrease in cultivation area and capital costs, 13% decrease in OPEX and 15% decrease in unit production costs.

The composition of the incoming wastewater (AD effluent from the abattoir's existing wastewater treatment plant) in terms of its nitrogen and phosphorous content is also an important parameter as it influences the amount of algae biomass that *stoichiometrically* can grow on the wastewater. By controlling the amount of biomass grown daily, the wastewater composition influences cultivation area, amount of final water effluent (through evaporation) and costs (Figure 12a and b). At Site D, the molar ratio between nitrogen and phosphorus in the incoming wastewater is very close to the Redfield stoichiometric ratio of 16:1. For this reason, an increase in N (or P) content doesn't impact on the system's performance as the growth of algae would be limited by the content of P (or N). Whether a decrease in N (or P) occurs, the maximum amount of algae that can grow daily on the wastewater would be proportionally limited by N (or P), further causing a decrease in cultivation area, capital and operational costs (Figure 12a and b). A net 34% and 17% increase in the amount of final water effluent and unit production costs, respectively, occur as a consequence of a 50% decrease in N (or P).

A directly proportional relationship has been found between the variation of wastewater flowrate and the process variables (Figure 12c): an increase (or decrease) in the amount of wastewater sent daily to the algae pond generates an equal increase (or decrease) in daily algae production, pond area, nutrient-depleted water for recycle, total CAPEX and OPEX. As a ratio between costs and algae biomass, a lower impact of variation in the wastewater flow rate has been found on the algae unit production costs (Figure 12c). A directly proportional relationship exists between the variation of capital costs (i.e., pond construction, dewatering system and lumped CAPEX) and costs performance variables (i.e., capital, operational and unit production costs). A 100% increase in pond construction (i.e., about AUD 100,000 per hectare) generates an increase in capital, operational and unit production costs of about 34%, 22% and 24%, respectively (Figure 12e). Likewise, a 100% increase in the construction costs associated with the dewatering system generates an increase in capital, operational and unit production costs of about 12%, 8% and 9%, respectively (Figure 12e). Obviously, variations of the cost parameters do not influence algae production, pond area and water effluent, which were omitted from graphs e, f and g of Figure 12.

The results of the sensitivity analysis on Site G are similar to Site D's ones and summarized in Figure 13. The same relationship for Site G and D is found between wastewater flow rate, energy costs and capital cost elements with the system's performance. The algae productivity per unit pond area is the most influential parameter for Site G too: its 50% decrease causes a 90% increase in cultivation area and capital costs, a 57% increase in OPEX and 64% increase in unit production costs (Figure 13d). Augmented algae productivity by 50% causes a 34% decrease in cultivation area and capital costs, 21% decrease in OPEX and 24% decrease in unit production costs (Figure 13d). Energy costs associated with pond mixing and dewatering systems don't impact on overall costs, whilst a directly proportional relationship has been found between the variation of wastewater flowrate and capital costs with the main performance variables (Figure 13).

Similar to Site D, the stoichiometric ratio between N and P in the incoming wastewater is the main parameter controlling the amount of algae biomass that can grow on the wastewater (Figure 13a and b). At a P content less than its base case value, the growth of algae is limited and controlled by phosphorous which causes a decreasing trend in daily production, cultivation area, capital and operational costs (Figure 13b). When nitrogen in the wastewater increases by 25 to 50%, phosphorous becomes the nutrient responsible of controlling the daily amount of algae production, which in turn impacts on land area and system costs (Figure 13a).

The overall variability of the selected process variables (i.e., daily algae production, pond area, nutrient-depleted water for recycle, total CAPEX, total OPEX and algae unit production cost) associated with the uncertainty of the modelling assumptions is summarized in Table 15. Within a -50% to +100% uncertainty associated with the modelling assumptions, the capital costs at Site D are characterized by an average and standard deviation of M$ 3.9 ± 1.1, with a maximum value of M$ 7.4 associated with an algae productivity as low as 13 $g/m^2/d$. Site G CAPEX have an average and standard deviation of M$ 2.1 ± 0.6, with a maximum value of M$ 4. OPEX ranges as M$ 2.1 ± 0.4 at Site D and M$ 1.3 ± 0.2 at Site G.

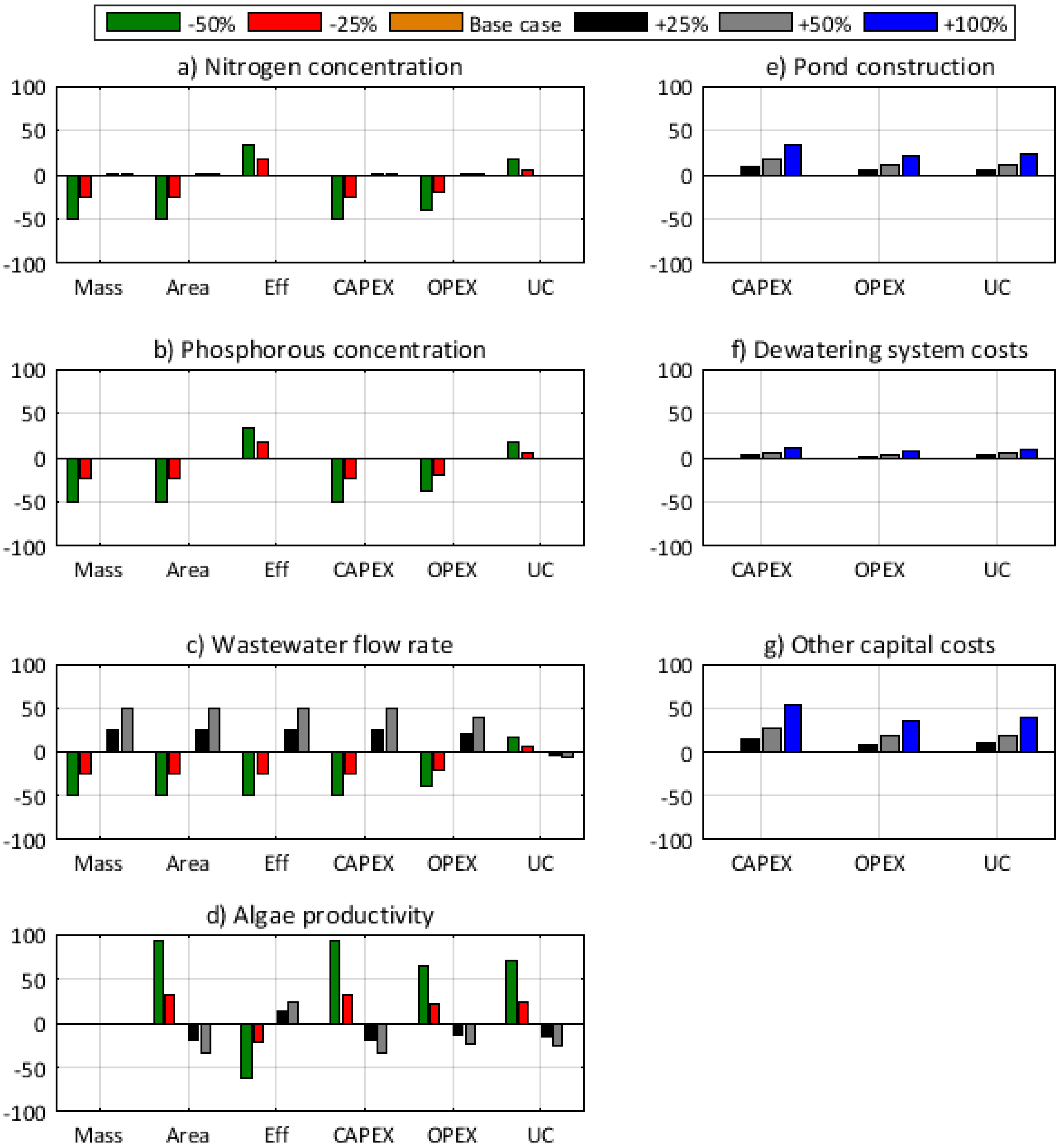


Figure 12. Sensitivity analysis on Site D. Percentage of variation of some process variables relative to the sensitivity of a) nitrogen content in the wastewater, b) phosphorous content in the wastewater, c) wastewater flow rate, d) algae productivity per unit area, e) raceway pond construction costs, f) dewatering system construction costs, g) other capital costs. Process variables are: Mass = daily grown algae biomass; Area = size of raceway ponds; Eff = nutrient-depleted water effluent; CAPEX = capital costs; OPEX =operational costs; UC = algae unit production costs.

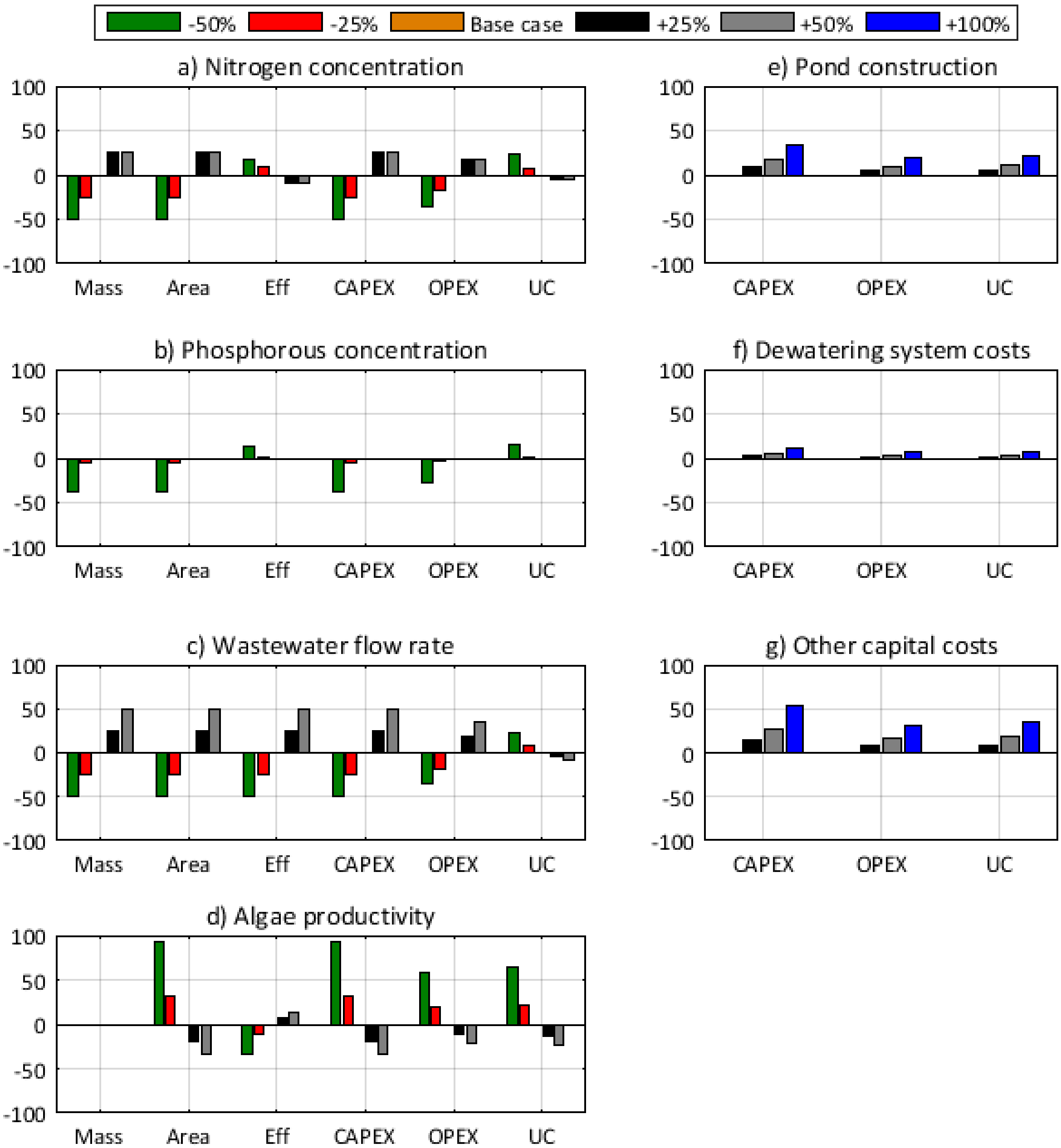


Figure 13. Sensitivity analysis on Site G. Percentage of variation of some process variables relative to the sensitivity of a) nitrogen content in the wastewater, b) phosphorous content in the wastewater, c) wastewater flow rate, d) algae productivity per unit area, e) raceway pond construction costs, f) dewatering system construction costs, g) other capital costs. Process variables are: Mass = daily grown algae biomass; Area = size of raceway ponds; Eff = nutrient-depleted water effluent; CAPEX = capital costs; OPEX =operational costs; UC = algae unit production costs.

Table 15. Summary of sensitivity analysis results – overall variability of major process variables.

	Base case ± standard deviation	Minimum	Maximum
Site D			
Daily algae production (kg/d)	5,221 ± 933	2,611	7,832
Pond area (ha)	21 ± 5	10	40
Final water effluent (kL/d)	1,276 ± 249	486	1,914
CAPEX (M AUD)	3.9 ± 1.1	1.9	7.4
OPEX (M AUD)	2.1 ± 0.4	1.3	3.5
Unit costs (AUD/ kg of algae)	1.55 ± 0.24	1.15	2.63
Site G			
Daily algae production (kg/d)	2,802 ± 502	1,401	4,203
Pond area (ha)	11 ± 3	6	22
Final water effluent (kL/d)	1,281 ± 164	641	1,922
CAPEX (M AUD)	2.1 ± 0.6	1.0	4.0
OPEX (M AUD)	1.3 ± 0.2	0.8	2.0
Unit costs (AUD/ kg of algae)	1.70 ± 0.25	1.3	2.8

6.0 DISCUSSION

The current project is structured into two main parts addressing a variety of deliverables. The first part is based on an extensive review of:

- The red meat processing industry and its current wastewater practice;
- Regulation standards relative to on-site water reuse and recycle;
- Data collection and analysis of wastewater operations at several Australian abattoirs;
- Integration of microalgae cultivation methods for wastewater treatment;
- Identification of wastewater streams generated at the reviewed abattoirs that are suitable for integration with a microalgae cultivation system.

Once specific abattoirs and wastewater streams have been selected, the techno-economic analysis of the proposed integrated microalgae cultivation system is performed as:

- Development of mass and energy balances of the integrated microalgae cultivation system for each selected abattoir;
- Environmental impact and risk assessment following potential on-site recycle of treated wastewater;
- Economic analysis of the proposed system;
- Sensitivity and uncertainty analysis of the developed techno-economic model.

The following discussion is mostly focused on the outcomes of the techno-economic analysis calculated at two abattoirs, i.e., Site D and Site G, as the most suitable abattoirs due to their comprehensive available set of data and the suitability of the wastewater effluent currently treated on site by anaerobic digestion to be integrated with a microalgae cultivation system. The outcomes and discussion relative to the literature review and data collection are addressed in Sections 5.1 to 5.3 and Appendices 9.1 to 9.3.

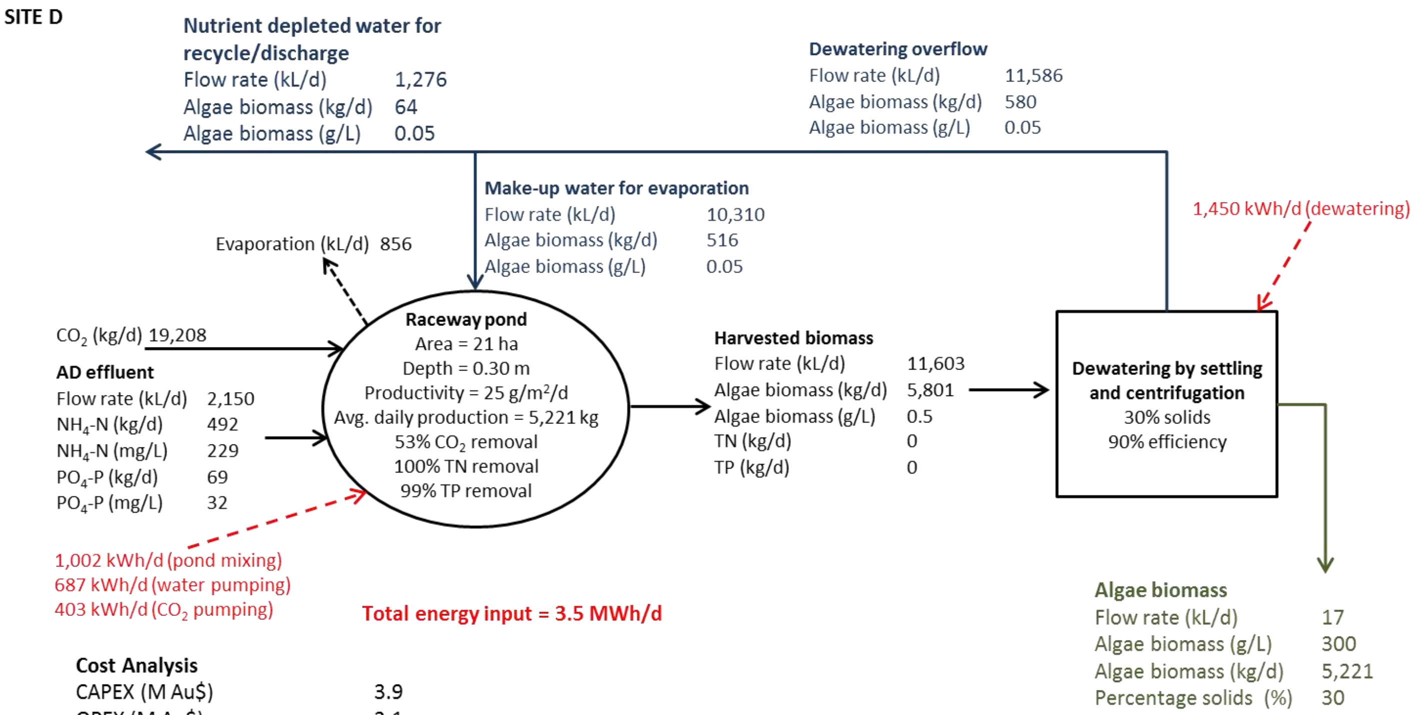
The mass balances on Site D and G (Figure 5 and 6) estimate the mass of algae biomass produced daily at 30% solids by the microalgae cultivation and dewatering system. The value associated with the microalgae product depends on its final use which, in turn, is to be determined by on-site needs (e.g., fertilizer, animal feed, biogas production) and the local market for algal products (e.g., oil extraction and biodiesel production, off-site animal feed). The environmental and process benefits associated with the recycle of the biomass to the AD process include augmented production of methane, which improves the economic and energy footprint of the abattoir and reduces its greenhouse gases emissions. Similarly, the use of biomass as on-site animal feed and/or fertilizer improves the overall environmental and economic footprint of the abattoir. Mass and energy balances have been developed at Sites D and G for the case where the microalgae biomass is used in an anaerobic digestion process to produce biogas. Although the amount of energy generated by AD of algae biomass offsets the energy demand of the integrated AD-microalgae cultivation process, increased capital and operational costs as well as increased process complexity and nutrient concentration in the final water effluent suggest the process without AD of the algae biomass is the preferred option for the reviewed abattoirs.

From an energy perspective, the typical energy consumption of an abattoir is reported in two MLA environmental performance review reports at 3,000 MJ/tHSCW (Ridoutt et al., 2015; URS, 2005) and 500 MJ/head (URS, 2005). It should be noted the range found in the literature for energy consumption expressed per animal head is very wide, and a value between 500 and 900 MJ/head is expected for abattoirs processing large animals. Site D processes an average of 1,100 heads (cattle and veal) per day (Jensen and Batstone, 2013) whilst about 500 heads are processed at Site G on a daily basis. Considering an average energy consumption of 500 MJ/head, an energy consumption of 550 GJ/d and 250 GJ/d is calculated at Site D and G, respectively (equivalent to about 152 MWh/d and 69 MWh/d for Site D and G, respectively). The daily energy consumption of the microalgae system estimated at Site D is 3.5 MWh/d (Table 10), which represents only 2.3% of the total daily energy consumption of the abattoir. Similarly for Site G, the daily energy consumption of the microalgae system is about 2.6% of the total energy demand of the abattoir.

The recommended system, its capital and operational costs together with the variability of the main process variables as a result of the sensitivity analysis is summarized in Figures 14 and 15 for Site D and Site G, respectively. A nutrient-depleted wastewater effluent of about 1,300 kL/d generated at both Sites D and G is a high-value process output. Subjected to water recycling guidelines implemented at each abattoir, the nutrient-depleted wastewater effluent can be recycled within the abattoir operations for all the permitted uses (e.g., cleaning of yards, infrastructures and trucks, washing of animals other than final wash, animal drinking water, fire control, irrigation of gardens and green areas) or discharged off-site for irrigation of crops and pasture or to surface water bodies. It should be noted that the primary objective of integrating a microalgae cultivation step in the wastewater treatment process currently operating at abattoirs is to reduce the environmental impacts associated with high nitrogen and phosphorus concentrations in the treated wastewater effluents currently generated in abattoirs and stored in open ponds. In this project it was demonstrated that a microalgae cultivation system treating the AD effluent is a very promising technology to achieve such objective. The production of a nutrient-depleted water stream that can be recycled within the abattoir operations and/or safely discharged to the environment is the main end-product of the suggested process. The potential recycle of a nutrient-depleted water stream within the abattoir's operations reduces the need of fresh water, optimizes the water balance of the entire facility and improves the self-sustainability and environmental footprint of the abattoir in regard to its water use.

The grown algae biomass represents a process by-product whose intrinsic value can be exploited as a way to offset some treatment costs and possibly produce a revenue stream. The final use of the concentrated microalgae biomass has to be evaluated on a case-by-case basis depending on the location and needs of the abattoir, local market for algae products, costs of handling and transportation of the biomass. Amongst its possible uses, on-site uses such as fertilizer for crop irrigation and use as animal feed are technically feasible and cost effective options. Off-site uses such as sale as animal feed, pharmaceutical products and biofuel generation could also be possible depending on the local market and the grown algal strain, although further drying of the biomass to achieve a 90% solids product might be necessary. The use of microalgae biomass (in particular *Chlorella* species) as crops fertilizer has shown to lead to positive effects on the environment in terms of the health of soils and plants. Similarly, the use of microalgae as animal feed has shown great potential in recent studies (Benemann, 2013), with *Chlorella* being one of the most robust and versatile species in the market. The use of algae biomass as protein supplement in animal feed has been widely suggested and researched as a sustainable way to help address the global energy, food and environmental crisis and the current competition for resources (e.g., land and water) between animal feed and human food supply (Benemann, 2013; Lum et al., 2013; Bleakley and Hayes, 2017). Recent estimates indicate that 30% of the global algal production is used by the animal feed industry and, although their nutritional profiles vary considerably with the species used, a large majority is characterized by protein, carbohydrate, and lipid contents that are comparable, if not superior, to conventional feeds (Lum et al., 2013). Recent estimates of the market value of *Chlorella* range from AUD 5 to AUD 20 per kg of algae, with the maximum of AUD 20 being associated with a 90% dry final product (Benemann, 2013). Considering the estimated algae production unit cost at Site D and Site G is about AUD 1.5-1.7 ± 0.25 per kg of grown algae (base case value ± standard deviation based on sensitivity analysis), there is a promising opportunity for the red meat processing industry to make the microalgae cultivation system extremely cost-effective and generate potential revenue by selling the grown biomass product as animal feed.

SITE D



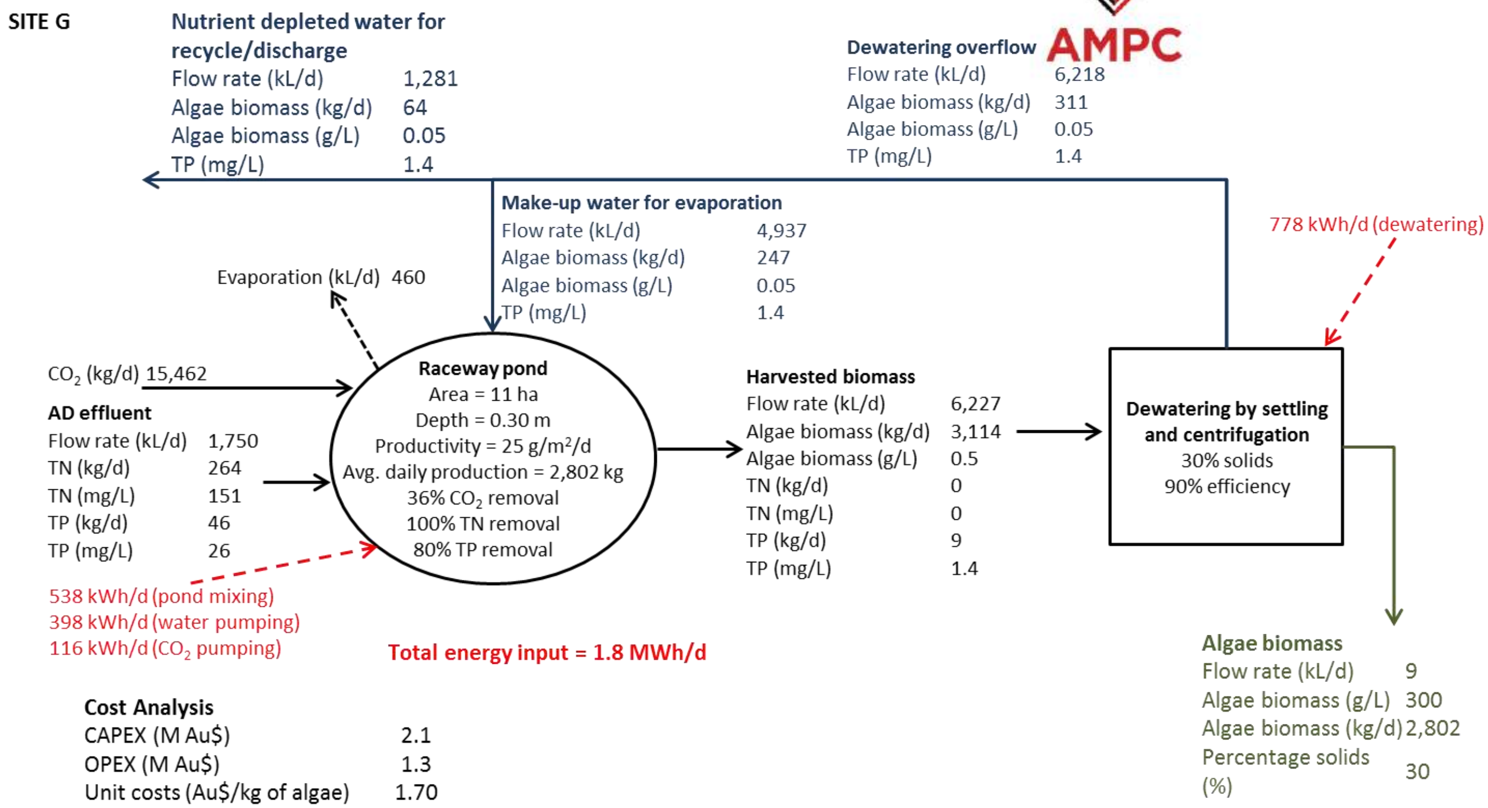
Cost Analysis

CAPEX (M Au$)	3.9
OPEX (M Au$)	2.1
Unit costs (Au$/kg of algae)	1.55

Summary of sensitivity analysis	Base case ± standard deviation	Minimum	Maximum
Daily algae production (kg/d)	5,221 ± 933	2,611	7,832
Pond area (ha)	21 ± 5	10	40
Final water effluent (kL/d)	1,276 ± 249	486	1,914
CAPEX (M Au$)	3.9 ± 1.1	1.9	7.4
OPEX (M Au$)	2.1 ± 0.4	1.3	3.5
Unit costs (Au$/ kg of algae)	1.55 ± 0.24	1.15	2.63

Wastewater streams
Clean water streams
Algae biomass product
Energy demand

Figure 14. Summary of the microalgae cultivation system proposed at Site D.



Summary of sensitivity analysis	Base case ± standard deviation	Minimum	Maximum
Daily algae production (kg/d)	2,802 ± 502	1,401	4,203
Pond area (ha)	11 ± 3	6	22
Final water effluent (kL/d)	1,281 ± 164	641	1,922
CAPEX (M Au$)	2.1 ± 0.6	1.0	4.0
OPEX (M Au$)	1.3 ± 0.2	0.8	2.0
Unit costs (Au$/ kg of algae)	1.70 ± 0.25	1.3	2.8

Wastewater streams
Clean water streams
Algae biomass product
Energy demand

Figure 15. Summary of the microalgae cultivation system proposed at Site G.

7.0 CONCLUSIONS/RECOMMENDATIONS

Overarching objective of this project is to investigate a new technological approach for the transformation of nutrient contents in red meat processing wastewater streams into protein rich biomass via microalgae cultivation. To address this objective, a techno-economic feasibility study of an integrated microalgae cultivation process for treating abattoir wastewaters and for water recycling has been developed. A thorough literature review has demonstrated the maturity and robustness of microalgae cultivation as a cost-effective treatment technology able to capture nutrient and harvest algae biomass whilst producing a nutrient-depleted water effluent. Through the development of a mathematical model, the output of a microalgae cultivation system integrated on the AD effluents currently generated at two Australian abattoirs has been quantified. The main outcomes of the modelling exercise are summarized as follow:

- A microalgae cultivation system that receives 2,000 kL/d of AD effluent at an average concentration of nitrogen and phosphorous ranging as 150-250 mg/L and 25-35 mg/L, respectively, is expected to generate about 1,300 kL/d of nutrient-depleted water suitable for recycle/discharge and about 3 to 5 tons of algae biomass product at 30% solids;
- A 80% to 100% nutrient removal from the AD effluent and fixation of about 6 to 10 tons/d of carbon dioxide into algae biomass are quantified;
- The cost-benefit analysis has estimated a range of capital and operational costs of M$ 2-4 and M$ 1-2, respectively, for an open raceway pond algae cultivation system followed by settling and centrifugation for algae harvesting and dewatering;
- Algae unit production costs are expected to range within $ 1.5 to 2 per kg of algae product.

Subjected to water recycling guidelines implemented at each abattoir, the nutrient-depleted water stream can be recycled within the abattoir operations thus improving the environmental impact of the abattoir and reducing costs associated with purchase of freshwater. Suggested uses of the effluent from microalgae cultivation refer to cleaning of yards, infrastructures and trucks, washing of animals other than final wash, animal drinking water, fire control, irrigation of gardens and green areas, irrigation of crops and pasture for fodder production. If recycle is not viable, the clean water stream is likely to meet the guidelines for safe discharge into the sewer or surface waters. The grown algae biomass represents a process by-product whose intrinsic value can be exploited to offset some treatment costs and possibly produce a revenue stream. On-site as fertilizer and animal feed are technically feasible and cost effective options.

The integrated microalgae cultivation system mitigates, and possibly removes, the environmental impacts associated with contamination of soils, water and groundwater, and to greenhouse gas emissions into the air. More importantly, it gives value to the wastewater effluent by recovering the nutrients and water and ultimately improving the environmental footprint of the abattoir. Several positive impacts of the microalgae cultivation process integrated on the AD effluent are identified:

- Reclamation of the environmental and economic value of the wastewater effluent in terms of water and nutrient recovery;
- Recycle of large volumes of water for on-site and off-site uses;

- Generation of an algae biomass product that is suitable for on-site reuse, energy generation or for sale to available markets;
- Sequestration of carbon dioxide currently generated by AD reactors during the current wastewater treatment process and fixation into algae biomass.

The promising outcomes of this technology assessment pave the road for further and more detailed explorations that aim at improving technical understanding, environmental and economic footprint and potential for full-scale applications. Experimental test works on microalgae cultivation on abattoir wastewaters are deemed essential to corroborate, improve and expand the techno-economic assessment developed in this project. Recommendations for future R&D projects include bioprospecting studies through a pilot plant implemented at the premises of an operating Australian abattoir. Through long-term outdoor experiments, microalgae species suitable for treating abattoir wastewaters are identified, together with the most suitable cultivation and harvesting methods, impacts of seasonal variations of inflow streams and nutrient supplies on algae growth rates. The optimization of microalgae growth conditions for maximum nutrient removal and the evaluation of different microalgae sources for animal/aquaculture feed or high value products is also an expected outcome of a bioprospecting study. Due to the high importance of algae productivity per pond square meter and AD effluent composition (flow rate, nitrogen and phosphorous content), it is recommended to carefully address the impacts of such parameters through ad-hoc lab and pilot experimental test work.

8.0 BIBLIOGRAPHY

Abou-Shanab, R.A., Ji, M.K., Kim, H.C., Paeng, K.J. and Jeon, B.H. (2013). Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. *Journal of Environmental Management*, 115, pp. 257-264.

AGWR, (2006). *Australian guidelines for water recycling: managing health and environmental risks (phase 1). National water quality management strategy*. Canberra: Environment Protection and Heritage Council, the Natural Resource Management Ministerial Council and the Australian Health Ministers' Conference.

Alcántara, C., García-Encina, P.A. and Muñoz, R. (2013). Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process. *Chemical engineering journal*, 221, pp.238-246.

AMPC, (2015). *Annual Report to Members 2014-2015*. [online]. Syndey: AMPC. Available at: http://www.ampc.com.au/uploads/pdf/annual-reports/2014-15-AnnualReport.pdf [Accessed 21 Oct. 2016].

ANZECC, (2000). *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*. Canberra: National Water Quality Management Strategy, ANZECC and ARMCANZ.

AQIS Meat Notice (2008). *Efficient use of water in export meat establishments*. Canberra: Department of Agriculture, Fisheries and Forestry. Available at: http://www.agriculture.gov.au/SiteCollectionDocuments/biosecurity/export/meat/elmer-3/notices/2008/2008-06.pdf [Accessed 21 Oct. 2016].

Ayre, J. (2013). *Microalgae culture to treat piggery anaerobic digestion effluent*. Doctoral dissertation, Honours thesis, Murdoch University, Perth. [online]. Available at: http://porkcrc.com.au/wp-content/uploads/2014/05/140420-Thesis-J-Ayre.pdf [Accessed 27 Jan. 2017].

Ayre, J.M., Moheimani, N.R. and Borowitzka, M.A. (2017). Growth of microalgae on undiluted anaerobic digestate of piggery effluent with high ammonium concentrations. *Algal Research*, 24, pp. 218-226.

Bazrafshan, E., Kord Mostafapour, F., Farzadkia, M., Ownagh, K.A. and Mahvi, A.H. (2012). Slaughterhouse Wastewater Treatment by Combined Chemical Coagulation and Electrocoagulation Process. *PLoS ONE*, 7(6): e40108.

Benemann, J. (2013). Microalgae for biofuels and animal feeds. *Energies*, 6(11), pp.5869-5886.

Benemann, J.R., Weissman, J.C., Koopman, B.L. and Oswald, W.J. (1977). Energy production by microbial photosynthesis. *Nature*, 268, pp. 19-23.

Benemann, J., Woertz, I. and Lundquist, T. (2012). Life cycle assessment for microalgae oil production. *Disruptive Science and Technology*, 1(2), pp.68-78.

Bleakley, S. and Hayes, M. (2017). Algal Proteins: Extraction, Application, and Challenges Concerning Production. *Foods*, 6(5), p.33.

Borowitzka, M.A. and Moheimani, N.R. (2013). Chapter 8 Open pond culture systems. *Algae for biofuels and energy*. Netherlands: Springer, pp. 133-152.

Browne, G. (2007). *Australian Standard for the Hygienic Production of Meat and Meat Products for Human Consumption (AS 4696-2007)*. Food Regulation Standing Committee Technical Report Series 3.

Bustillo-Lecompte, C.F. and Mehrvar, M. (2015). Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances. *Journal of Environmental Management*, 161, pp. 287-302.

Cai, T., Park, S.Y. and Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: status and prospects. *Renewable and Sustainable Energy Reviews*, 19, pp. 360-369.

Campbell, P.K., Beer, T. and Batten, D. (2011). Life cycle assessment of biodiesel production from microalgae in ponds. *Bioresource technology*, 102(1), pp. 50-56.

Chaudry, S., Bahri, P.A. and Moheimani, N.R. (2017). Superstructure optimization and energetic feasibility analysis of process of repetitive extraction of hydrocarbons from Botryococcus braunii–a species of microalgae. *Computers & Chemical Engineering*, 97, pp. 36-46.

Clarens, A.F., Resurreccion, E.P., White, M.A. and Colosi, L.M. (2010). Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental science & technology*, 44(5), pp.1813-1819.

CPI website. Available at: http://www.usinflationcalculator.com/inflation/consumer-price-index-and-annual-percent-changes-from-1913-to-2008/ [Accessed 04 Sep. 2017].

Collet, P., Hélias, A., Lardon, L., Ras, M., Goy, R.A. and Steyer, J.P. (2011). Life-cycle assessment of microalgae culture coupled to biogas production. *Bioresource technology*, 102(1), pp.207-214.

COWI, (2000). *Cleaner production assessment in meat processing*. 1st ed. Paris: United Nations Environment Programme, Division of Technology, Industry and Economics; Copenhagen: Danish Environmental Protection Agency, c2000.

Craggs, R.J., McAuley, P.J. and Smith, V.J. (1997). Wastewater nutrient removal by marine microalgae grown on a corrugated raceway. *Water Research*, 31(7), pp. 1701-1707.

Craggs, R.J., Lundquist, T.J. and Benemann, J.R. (2013). Chapter 9 Wastewater Treatment and Algal Biofuel Production. *Algae for biofuels and energy*. Netherlands: Springer, pp. 153-163.

CSIRO, (2014). Water recycling in food production and manufacturing. Australian Water Recycling, Centre of Excellence, CSIRO.

Cuellar-Bermudez, S.P., Aleman-Nava, G.S., Chandra, R., Garcia-Perez, J.S., Contreras-Angulo, J.R., Markou, G., Muylaert, K., Rittmann, B.E. and Parra-Saldivar, R. (2017). Nutrients utilization and contaminants removal. A review of two approaches of algae and cyanobacteria in wastewater. *Algal Research*, 24, pp. 438-449.

De-Bashan, L.E., Hernandez, J.P., Morey, T. and Bashan, Y. (2004). Microalgae growth-promoting bacteria as "helpers" for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. *Water Research*, 38(2), pp. 466-474.

Export Orders, (2005). *Export Control (Meat and Meat Products) Orders 2005*. Canberra: Office of Parliamentary Counsel. Available at: https://www.legislation.gov.au/Series/F2005L00154 [Accessed 23 Nov. 2016].

Fallowfield, H.J. and Garrett, M.K. (1985). The photosynthetic treatment of pig slurry in temperate climatic conditions: a pilot-plant study. *Agricultural Wastes*, 12(2), pp. 111-136.

Food Act, (2008). *Food Act Western Australia*. Available at: https://www.slp.wa.gov.au/pco/prod/FileStore.nsf/Documents/MRDocument:7633P/$FILE/FoodAct2008_00-00-01.pdf?OpenElement [Accessed 23 Nov. 2016].

Ford, (2013). *Facilitation of water reuse projects*. [online]. Sydney: AMPC. Available at: http://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Environment-Off-Farm/Water-efficiency-reuse-and-recycling/1565 [Accessed 28 Oct. 2016].

FSANZ, (2015a). *Standard 4.2.3 Production and processing standard for meat*. [online]. Canberra: Food Standards Australia New Zealand. Available at: http://www.foodstandards.gov.au/code/primaryproduction/meatandmeatproducts/Pages/default.aspx [Accessed 11 Nov. 2016].

FSANZ, (2015b). *Supporting document 5 Food safety management in the meat industry Proposal P1014 Primary Production & Processing Standard for Meat & Meat Products*. [online]. Canberra: Food Standards Australia New Zealand. Available at: http://www.foodstandards.gov.au/code/primaryproduction/meatandmeatproducts/Pages/default.aspx [Accessed 11 Nov. 2016].

Garden, D. (2005). *Wastewater HRP algae production for biofuel*. [online]. Available at: http://www.civil.canterbury.ac.nz/cochrane/NREProjects/Danny_Garden_Final_Report.pdf [Accessed 27 Jan. 2017].

Ghayal, M.S. and Pandya, M.T. (2013). Microalgae biomass: a renewable source of energy. *Energy Procedia* , 32, pp.242-250.

Hamawand, I. (2015). *Review of wastewater treatment chemicals and organic chemistry alternatives for abattoir effluent*. [online]. Syndey: AMPC. Available at: http://www.ampc.com.au/2015/02/Review-of-wastewater-treatment-chemicals-and-organic-chemistry-alternatives-for-abattoir-effluent [Accessed 21 Oct. 2016].

Handler, R.M., Canter, C.E., Kalnes, T.N., Lupton, F.S., Kholiqov, O., Shonnard, D.R. and Blowers, P. (2012). Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: Analysis of the prior literature and investigation of wide variance in predicted impacts. *Algal Research*, 1(1), pp.83-92.

Hernández, D., Riaño, B., Coca, M. and García-González, M.C. (2013). Treatment of agro-industrial wastewater using microalgae–bacteria consortium combined with anaerobic digestion of the produced biomass. *Bioresource Technology*, 135, pp. 598-603.

Hernández, D., Riaño, B., Coca, M., Solana, M., Bertucco, A. and García-González, M.C. (2016). Microalgae cultivation in high rate algal ponds using slaughterhouse wastewater for biofuel applications. *Chemical Engineering Journal*, 285, pp. 449-458.

Jebali, A., Acién, F.G., Gómez, C., Fernández-Sevilla, J.M., Mhiri, N., Karray, F., Dhouib, A., Molina-Grima, E. and Sayadi, S. (2015). Selection of native Tunisian microalgae for simultaneous wastewater treatment and biofuel production. *Bioresource Technology*, 198, pp. 424-430.

Jensen, P., Huelsen, T., Batstone, D. and Keller, J. (2013). Review and evaluation of the application of anaerobic ammonium removal technology for wastewater treatment. A.ENV.0162. Final report. [online]. Syndey: AMPC. [Accessed 11 Aug. 2017].

Jensen, P. (2015). *Nutrient recovery from paunch and covered anaerobic lagoon effluent*. [online]. Syndey: AMPC. Available at: https://www.ampc.com.au/uploads/cgblog/id85/2013_4007-Nutrient-recovery-from-paunch.pdf. [Accessed 5 May 2017].

Jensen and Batstone, (2012). *Energy and Nutrient analysis on Individual Waste Streams*. [online]. Syndey: AMPC. Available at: http://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Environment-Off-Farm/Wastewater-treatment-and-nutrient-removal/1563 [Accessed 28 Oct. 2016].

Jensen and Batstone, (2013). *NGERS and Wastewater Management – mapping waste streams and quantifying the impacts*. [online]. Syndey: AMPC. Available at: http://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Environment-Off-Farm/Wastewater-treatment-and-nutrient-removal/1563 [Accessed 28 Oct. 2016].

Laginestra, M. (2012). *Covered anaerobic lagoons*. Final report. A.ENV.0135. Published by Meat and Livestock Australia Limited. Syndey, July 2012.

Lum, K.K., Kim, J. and Lei, X.G. (2013). Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *Journal of animal science and biotechnology*, 4(1), p.53.

Lundquist, T.J., Woertz, I.C., Quinn, N.W.T. and Benemann, J.R. (2010). A realistic technology and engineering assessment of algae biofuel production. *Energy Biosciences Institute*, p.1.

Maroneze, M.M., Barin, J.S., Menezes, C.R.D., Queiroz, M.I., Zepka, L.Q. and Jacob-Lopes, E. (2014). Treatment of cattle-slaughterhouse wastewater and the reuse of sludge for biodiesel production by microalgal heterotrophic bioreactors. *Scientia Agricola*, 71(6), pp. 521-524.

Matheyarasu, R., Seshadri, B., Bolan, N.S. and Naidu, R. (2015). Impacts of abattoir waste-water irrigation on soil fertility and productivity. In *Irrigation and Drainage-Sustainable Strategies and Systems*. InTech.

Mennaa, F.Z., Arbib, Z. and Perales, J.A. (2015). Urban wastewater treatment by seven species of microalgae and an algal bloom: biomass production, N and P removal kinetics and harvestability. *Water Research*, 83, pp. 42-51.

Mittal, G.S. (2006). Treatment of wastewater from abattoirs before land application—a review. *Bioresource Technology*, 97(9), pp. 1119-1135.

Moheimani, N.R. (2005). The culture of coccolithophorid algae for carbon dioxide bioremediation. Doctoral dissertation, Murdoch University, Perth.

Molinuevo-Salces, B., Mahdy, A., Ballesteros, M. and González-Fernández, C. (2016). From piggery wastewater nutrients to biogas: Microalgae biomass revalorization through anaerobic digestion. *Renewable Energy*, 96, pp. 1103-1110.

NHMRC, (2011). *Australian drinking water guidelines paper 6 national water quality management strategy*. National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia, Canberra.

Nwoba, E.G., Ayre, J.M., Moheimani, N.R., Ubi, B.E. and Ogbonna, J.C. (2016). Growth comparison of microalgae in tubular photobioreactor and open pond for treating anaerobic digestion piggery effluent. *Algal Research*, 17, pp. 268-276.

Park, J.B.K., Craggs, R.J. and Shilton, A.N. (2011). Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology*, 102(1), pp. 35-42.

Polakovičová, G., Kušnír, P., Nagyová, S. and Mikulec, J. (2012). Process integration of algae production and anaerobic digestion. In *15th international conference on process integration, modelling and* (Vol. 29).

Oswald, W.J. (2003). My sixty years in applied algology. *Journal of Applied Phycology*, 15(2-3), pp. 99-106.

Oswald, W.J. and Golueke, C.G. (1960). Biological transformation of solar energy. *Advances in applied microbiology*, 2, pp. 223–262.

Raes, E.J., Isdepsky, A., Muylaert, K., Borowitzka, M.A. and Moheimani, N.R. (2014). Comparison of growth of Tetraselmis in a tubular photobioreactor (Biocoil) and a raceway pond. *Journal of Applied Phycology*, 26(1), pp. 247-255.

Ras, M., Lardon, L., Bruno, S., Bernet, N. and Steyer, J.P. (2011). Experimental study on a coupled process of production and anaerobic digestion of Chlorella vulgaris. *Bioresource technology*, 102(1), pp.200-206.

Ridoutt, B., Sanguansri, P. and Alexander, D. (2015). *Environmental Performance Review: Red Meat Processing Sector 2015 FINAL REPORT*. [online]. Syndey: AMPC. Available at: http://www.ampc.com.au/uploads/cgblog/id23/Environmental-Performance-Review-Red-Meat-Processing-Sector-2015.pdf [Accessed 21 Oct. 2016].

Sander, K. and Murthy, G.S. (2010). Life cycle analysis of algae biodiesel. *The International Journal of Life Cycle Assessment*, 15(7), pp.704-714.

Sunder, G.C and Satyanarayan, S. (2013). Efficient Treatment of Slaughter House Wastewater by Anaerobic Hybrid Reactor Packed with Special Floating Media. *International Journal of Chemical and Physical Sciences*, 2, Special Issue - March 2013, pp. 73-81.

Taşkan, E. (2016). Performance of mixed algae for treatment of slaughterhouse wastewater and microbial community analysis. *Environmental Science and Pollution Research*, 23(20), pp. 20474-20482.

URS (2005). Industry environmental performance review. Integrated meat processing plants. PRENV.033. Published by Meat and Livestock Australia Limited. Syndey, April 2005.

Wang, M. and Park, C. (2015). Investigation of anaerobic digestion of Chlorella sp. and Micractinium sp. grown in high-nitrogen wastewater and their co-digestion with waste activated sludge. *Biomass and Bioenergy*, 80, pp.30-37.

Wang, M., Yang, Y., Chen, Z., Chen, Y., Wen, Y. and Chen, B. (2016). Removal of nutrients from undiluted anaerobically treated piggery wastewater by improved microalgae. *Bioresource Technology*, 222, pp. 130-138.

Warnecke, M., Farrugia, T. and Ferguson, C. (2008). Review of abattoir water usage reduction, recycling and reuse. A.PIA.0086. Final Report. [online]. Syndey: AMPC. Available at: http://www.ampc.com.au/2008/06/Review-of-abattoir-water-usage-reduction-recycling-and-reuse [Accessed 11 Aug. 2017].

Wijihastuti, R.S. (2017). Botryococcus braunii growth and photosynthetic activity in biofilm (Doctoral dissertation, Murdoch University).

Yuan, X., Wang, M., Park, C., Sahu, A.K. and Ergas, S.J. (2012). Microalgae growth using high-strength wastewater followed by anaerobic co-digestion. *Water Environment Research*, 84(5), pp.396-404.

Zhang, T.Y., Hu, H.Y., Wu, Y.H., Zhuang, L.L., Xu, X.Q., Wang, X.X. and Dao, G.H. (2016). Promising solutions to solve the bottlenecks in the large-scale cultivation of microalgae for biomass/bioenergy production. *Renewable and Sustainable Energy Reviews*, 60, pp. 1602-1614.

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P. and Yuan, Z. (2013). Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research*, 47(13), pp. 4294-4302.

Zittelli, G.C., Rodolfi, L., Bassi, N., Biondi, N. and Tredici, M.R. (2013). Chapter 7 Photobioreactors for microalgal biofuel production. *Algae for biofuels and energy*. Netherlands: Springer, pp. 115-131.

9.0 APPENDICES

9.1 Appendix 1. Reviewed Australian abattoirs

The following data on flowsheet and wastewater streams composition is sourced by two AMPC/MLA reports produced by Jensen and Batstone in 2012 and 2013. The authors interviewed and gathered data on wastewater source, composition, collection and treatment at six different Australian red meat processing facilities, hereafter referred to as Sites A to F. Extensive water quality monitoring and mass balances allowed the characterization of each wastewater stream, to eventually identify the most critical waste streams and recommend a proper treatment process. The level of detail of the collected data varies for each site, and the same information might not be available for all sites, thus leading to potential inconsistencies in the data reporting. As suggested by Jensen and Batstone (2013), Sites A, C and D provide the most accurate and comprehensive dataset, whilst the structure of the waste handling process at Sites B, E and F prevented the collection of data from some individual processing areas, thus the analysis of nutrient and organic loads is not as reliable as for Sites A, C and D.

The collection of data from another beef abattoir has started as part of the current project. The Australian beef abattoir is referred hereafter as Site G and communication between Murdoch University and Site G is considered strictly confidential. Although not as comprehensive as for Sites A, C and D, the information collected from Site G is used as a reference case study for the current project together with the sites analyzed by Jensen and Batstone (2012 and 2013). Site G is a large export-registered cattle meat processor, with a current throughput of 130,000 heads per annum and about 2,500 heads per week. About 70% of the meat produced at Site G is for export markets in Asia, Middle East and U.S., with the remaining 30% for domestic use supply.

9.1.1 Processing Site A

A flowsheet of wastewater sources, handling and treatment at Site A is shown in Figure A1.1. The composition of each wastewater stream is presented in Table A1.1. Data are based on beast weight of 600 kg. Raw data can be found in the original report by Jensen and Batstone (2012). The combined wastewater effluent is treated in an anaerobic pond. Streams generated by slaughtering, paunch processing, tripe and cattle wash undergo screening for solid separation prior to anaerobic digestion (AD). Wastewater streams generated by rendering and boning activities are treated by screening as well as saveall/dissolved air flotation (DAF) for further solid separation and collection of fat, oil and grease. The effluent from the DAF unit is sent to AD.

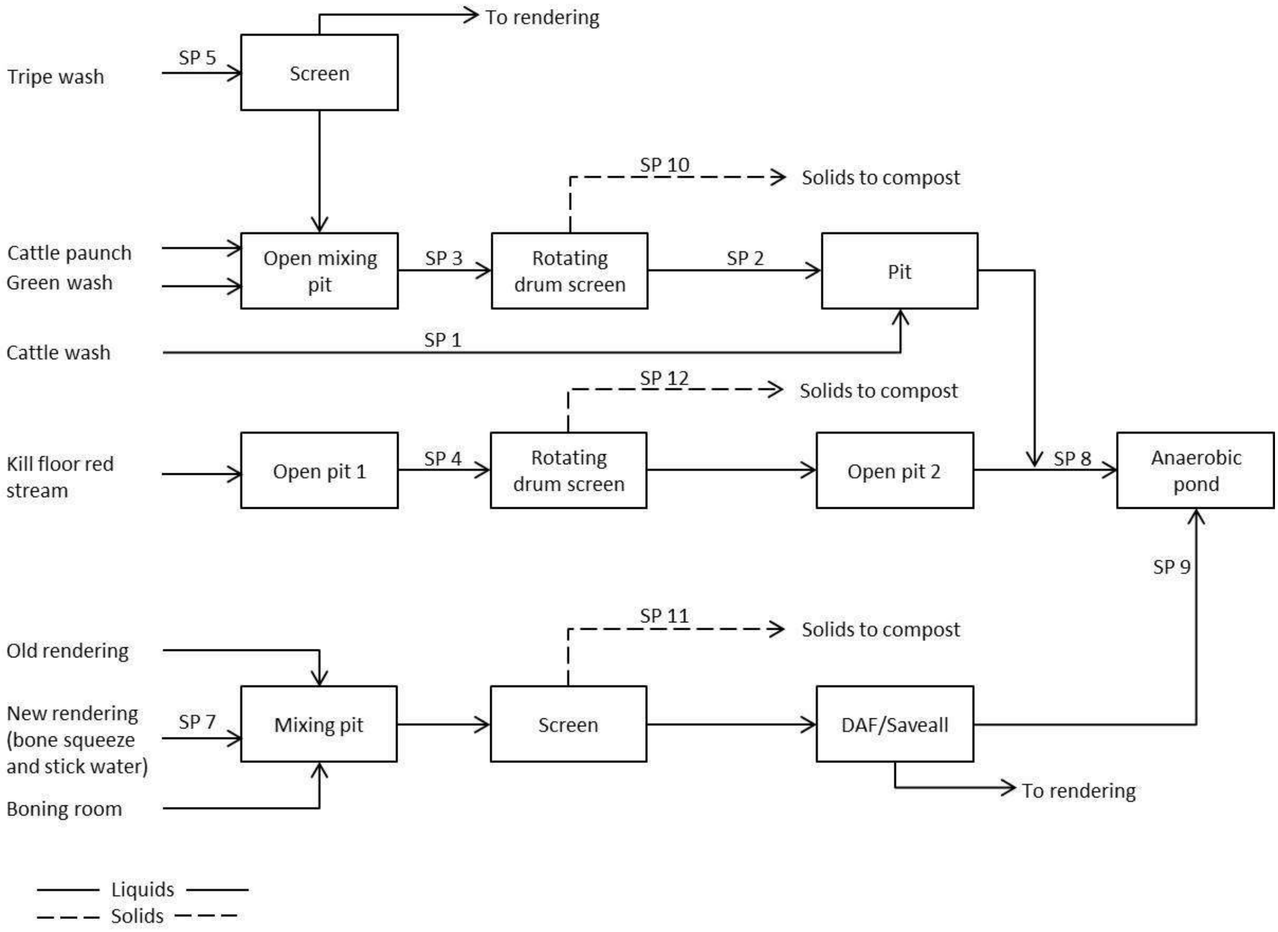


Figure A1.1. Flowsheet of wastewater sources, handling and treatment at Site A (adapted from Jensen and Batstone, 2012). SP: sample point.

Table A1.1. Characterization of wastewater streams at Site A. SP: sample point (refer to Figure A1.1).

Site A										
ID	Stream	Volume (kL/d)	TCOD (mg/L)	SCOD (mg/L)	TS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TKP (mg/L)	PO_4-P (mg/L)
SP 1	Cattle wash	882	3,194	380	3,000	4	89	47	13	6
SP 2	Paunch liquid	311	23,908	2,064	15,800	2,603	517	36	211	160
SP 3	Paunch, tripe, green wash	330	32,707	2,170	24,800	3,883	281	15	155	101
SP 4	Kill floor	450	3,756	1,278	3,500	206	2,021	17	28	17
SP 5	Tripe wash	54	30,890	1,210	19,900	11,638	282	9	81	43
SP 7	New Render	192	40,003	7,840	24,600	5,538	1,718	41	120	73
SP 8	Total effluent cold	1,512	16,378	1,798	10,600	3,063	234	67	77	75
SP 9	Total effluent hot	911	7,209	1,600	4,800	1,138	264	44	28	17
	Total effluent	2,423	12,893	1,722	8,396	2,332	245	58	58	53

9.1.2 Processing Site B

Site B is a mixed 50% beef and 50% sheep abattoir and data are based on weekly HSCW (hot standard carcass weight). Figure A1.2 shows the flowsheet highlighting source, collection and primary treatments of each wastewater stream at Site B. Jensen and Batstone (2012) could not separate all the streams at Site B, therefore some streams have combined beef and sheep data. The stream composition is shown in Table A1.2. Raw data can be found in the original report by Jensen and Batstone (2012).

Two process trains characterize the wastewater system at Site B. A combined red stream includes rendering, offal processing and boning, while cattle wash and sheep paunch and intestinal wash form a second stream. Both streams go through a solid separation phase, and are then combined in a Gross Fat Separator. The fat scarped from the top is sent to rendering, whilst the liquid is sent to a clarifier and a DAF unit for further separation of solids and fats. No biological treatments seem to occur at Site B (Figure A1.2), despite the high concentration of contaminants still present in the final total effluent (Table A1.2). No further details on the wastewater treatment processes are provided by Jensen and Batstone (2012).

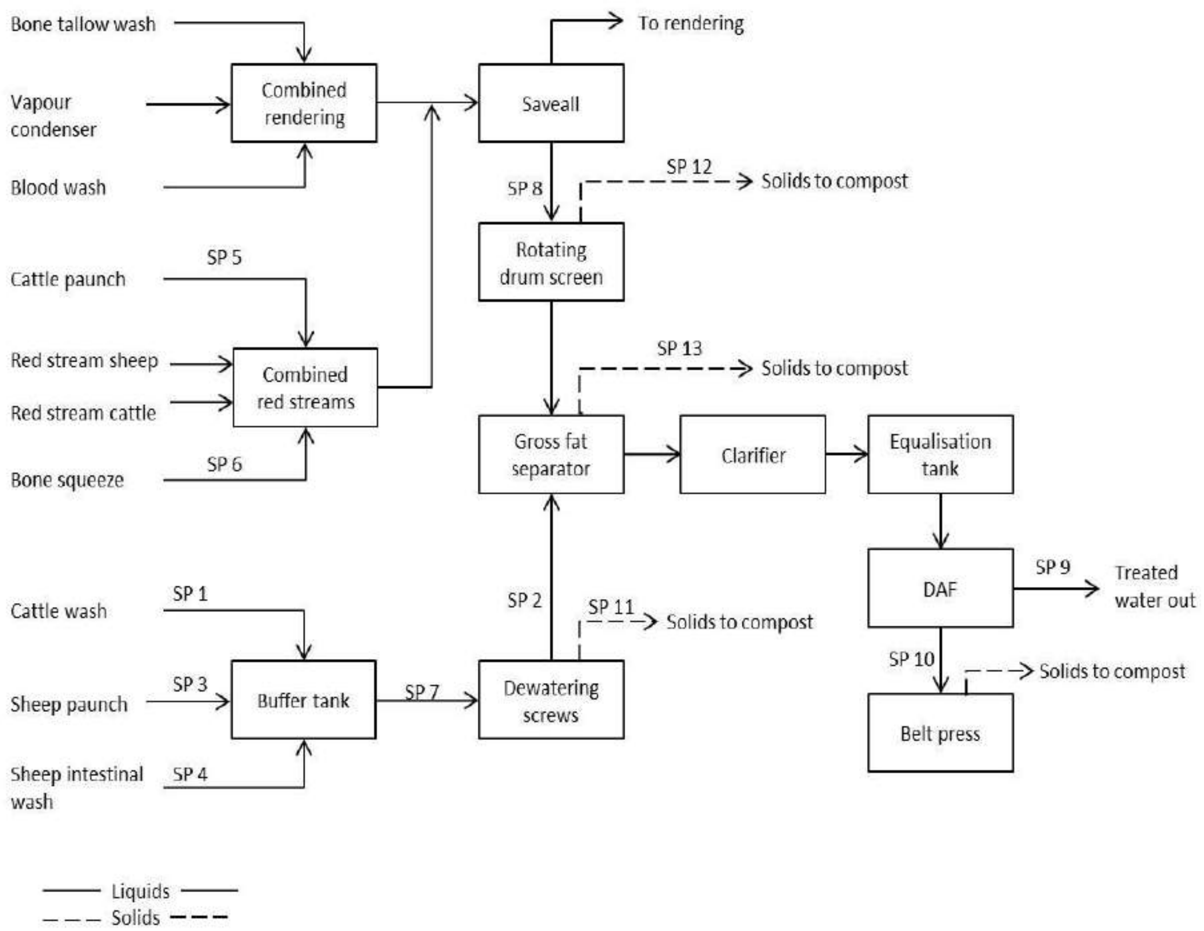


Figure A1.2. Flowsheet of wastewater sources, handling and treatment at Site B (adapted from Jensen and Batstone, 2012). SP: sample point.

Table A1.2. Characterization of wastewater streams at Site B. SP: sample point (refer to Figure A1.2).

Site B										
ID	Stream	Volume (kL/d)	TCOD (mg/L)	SCOD (mg/L)	TS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TKP (mg/L)	PO_4-P (mg/L)
SP 1	Cattle wash	252	3,089	534	3,450	4	220	131	40	20
SP 2	Paunch liquid	421	10,777	2,280	8,100	47	377	190	233	162
SP 3	Sheep paunch	60	52,663	4,890	55,410	226	1,685	181	1,805	922
SP 4	Sheep intestinal wash	120	5,285	1,900	4,550	30	125	103	35	30
SP 5	Cattle paunch	N/A	39,158	2,805	47,880	120	1,390	58	640	251
SP 6	Bone squeeze	285	44,773	-	33,200	25	4,745	131	11	34
SP 7	Buffer tank	400	13,877	2,124	16,900	29	674	197	314	149
SP 8	Saveall out	2,138	10,367	2,200	7,000	1,313	304	71	49	33
SP 9	DAF effluent	3,153	9,587	1,970	4,300	783	232	93	50	38
	Total effluent	3,153	9,587	1,970	4,300	783	232	93	50	38

9.1.3 Processing Site C

A flowsheet of wastewater sources, handling and treatment at Site C is shown in Figure A1.3. The composition of each wastewater stream is presented in Table A1.3. Each wastewater stream generated at each operation at Site C is collected in a mixed pit prior to AD. Solid screening occurs before the mixing with different screening processes for the cattle wash waters and the slaughtering/rendering produced wastewaters. Data in Table A1.3 are based on beast weight of 600 kg. Raw data can be found in the original report by Jensen and Batstone (2012).

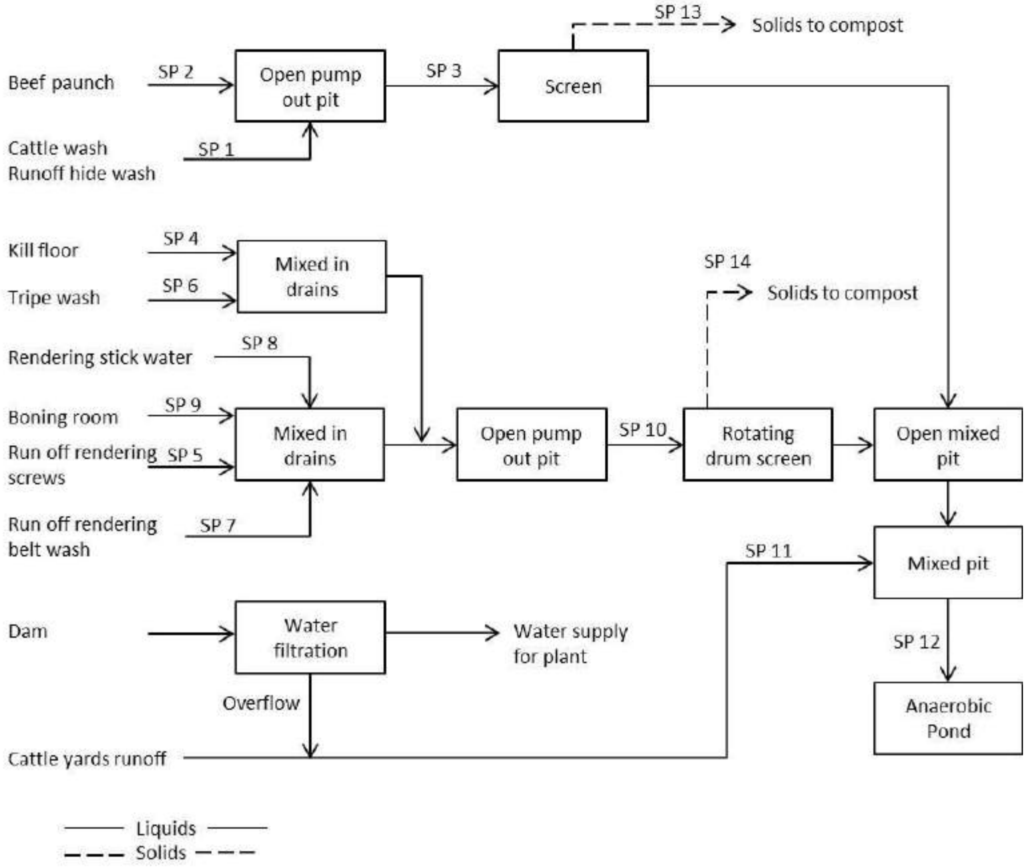


Figure A1.3. Flowsheet of wastewater sources, handling and treatment at Site C (adapted from Jensen and Batstone, 2012). SP: sample point.

Table A1.3. Characterization of wastewater streams at Site C. SP: sample point (refer to Figure A1.3).

Site C										
ID	Stream	Volume (kL/d)	TCOD (mg/L)	SCOD (mg/L)	TS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TKP (mg/L)	PO_4-P (mg/L)
SP 1	Cattle wash	240	1,632	680	2,250	< 1	175	82	26	14
SP 2	Paunch	200	15,028	2,096	13,370	210	506	46	256	112
SP 3	Green pit	440	5,768	774	5,350	217	276	43	96	41
SP 4	Kill room floor	108	19,257	7,380	7,290	28	3,040	41	57	20
SP 5	Screws to Rendering	21	24,490	9,900	19,240	1,717	3,050	252	417	145
SP 6	Tripe wash	432	10,392	428	2,870	687	51	6	24	13
SP 7	Rendering belt wash	25	6,903	692	4,850	3,430	164	1	19	8
SP 8	Stick water	315	22,103	2,400	13,070	6,017	718	21	108	51
SP 9	Boning room	90	-	-	340	< 1	-	-	-	-
SP 10	Red pit	949	9,683	1,324	6,190	4,400	258	10	24	14
SP 11	Cattle yards, clean overflow	171	-	-	190	-	-	-	-	-
SP 12	Total effluent	2,115	10,785	893	7,530	3,350	260	62	30	15

9.1.4 Processing Site D

Site D is situated in New South Wales, Australia, and has the capability to process 12,500 bovines per week. The abattoir has two separate processing floors one for beef and one for veal meat. The animals are grass/grain fed and the abattoir consumes 2.5-3 ML of clean water daily. Figure A1.4 shows the flowsheet of source, collection and primary treatments of each wastewater stream at Site D. Table A1.4 gives the composition of each stream analyzed by Jensen and Batstone (2013). Where available, composite samples were collected, otherwise an average composition of the available samples has been considered. Raw data and additional information can be found in the original report by Jensen and Batstone (2013).

The waste processing operations at the Site D abattoir consists of 4 main process trains (Figure A1.4):

- Combined red wastewater includes all wastewater from the rendering plant, beef slaughter floor, offal processing and the veal slaughter floor. The rendering plant includes several wastewater sources including raw material bins, stick waters, boiler condensate. The beef slaughter floor and offal processing have been collected as a combined stream. The combined red wastewater is passed through a screen to remove course solids (recycled to rendering) and sent to a DAF system with no polymer addition to recover fatty solids for recycling. The remaining red wastewater flows directly to the final effluent mixing pit and is discharged to the anaerobic lagoon.
- Paunch handling consists of paunch, foreign objects (e.g. intestinal plugs/clamps) and wash down water/transfer water. The combined paunch stream passes through a course screen and a screw press to remove coarse solids that are sent to composting. The remaining wastewater flows directly to the final effluent mixing pit and is discharged to the anaerobic lagoon.
- Cattle yards' wastewaters consist of spray water used to wash cattle before processing, bovine urine and manure, and wash down from cleaning operations in the cattle yards. A portion of water used in the cattle yards is recycled from the defrost collection pit. The preliminary treatment consists in a pass through an auger screw to remove coarse solids that are sent to composting. The remaining wastewater flows directly to the final effluent mixing pit and is discharged to the anaerobic lagoon.
- Boning room and chillers wastewaters are collected in the defrost collection pit. This wastewater is recycled to the cattle yards and is not directly discharged to the anaerobic lagoon.

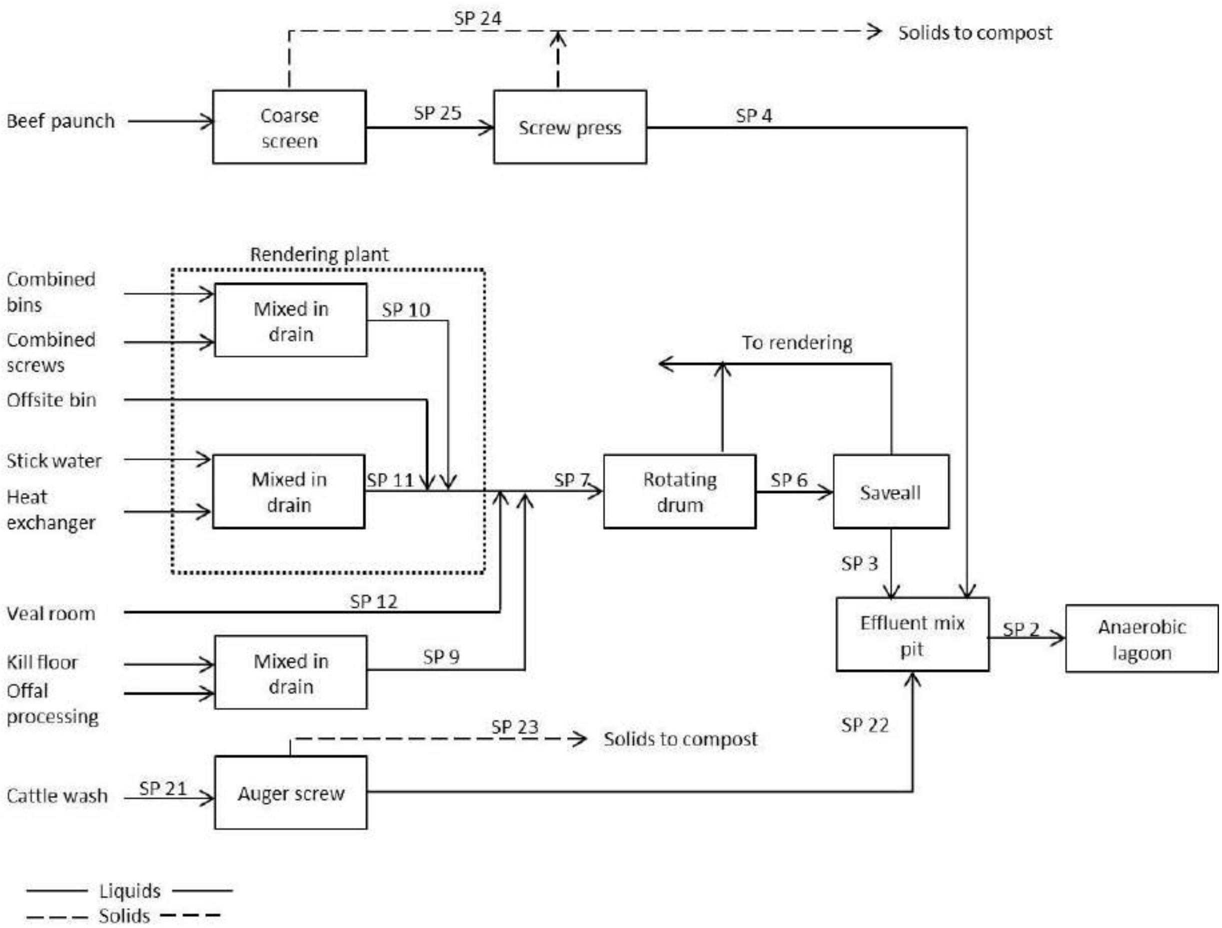


Figure A1.4. Flowsheet of wastewater sources, handling and treatment at Site D (adapted from Jensen and Batstone, 2013). SP: sample point.

9.1.5 Processing Site E

Site E is a beef only facility situated in Queensland, Australia. Site E processes grass fed, grain fed and organic beef, and has a capability of approximately 3,000 bovines per week. Site E consumes 1.5 ML of clean water daily. Figure A1.5 summarizes Site E flowsheet. The composition of each wastewater stream is presented in Table A1.5. Where available, composite samples were collected, otherwise an average composition of the available samples has been considered. Site E includes a red wastewater stream that combines streams from the slaughter floor, rendering plant and boning room. Site E also has a combined green wastewater stream generated from paunch handling, offal and cattle yards. The treatment at Site E is composed of preliminary screening and a DAF system to remove solids and FOG. Jensen and Batstone (2013) identified some issues happening in the plant during their site visit. Cattle wash was not operating during the sample period, thus the characterization of wastewater coming from the cattle yards is not representative of standard operations. Site E transports cattle hides to a fleshing shed using a water slide system, thus resulting in an additional wastewater stream that is added to the combined red wastewater. Slaughter floor and boning room wastewater contribute to the combined red flow, but these streams were not accessible during the sample trip, thus no data have been measured.

Table A1.4. Characterization of wastewater streams at Site D. SP: sample point (refer to Figure A1.4).

Site D									
ID	Stream	Volume (kL/d)	Temp (°C)	TCOD (mg/L)	SCOD (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	FOG (mg/L)
25	Paunch - pre screen	200	33	12,190	920	15,123	12,897	N/A	142
4	Paunch - post screen	200	34	5,420	850	6,946	4,753	4,370	194
24	Paunch - solids	18 m3	N/A	147,170	N/A	249,383	236,614	N/A	1,095
21	Cattle wash - pre auger	400	21	11,070	400	9,828	7,940	N/A	82
22	Cattle wash - post auger	400	19	1,800	250	1,979	1,361	1,200	10
23	Cattle wash - solids	2 m3	N/A	89,530	N/A	155,983	136,101	N/A	380
10	Combined bins	304	46	44,140	15,820	30,548	26,376	17,730	9,297
11	Combined stick	94	39	73,420	980	33,530	32,130	32,030	21,075
12	Veal room	480	31	14,120	2,270	9,335	8,942	276	< 4
9	Kill floor/offal	722	37	2,210	1,220	2,630	2,245	2,020	325
7	Combined red - pre screen	1,600	38	9,950	1,910	8,489	7,827	5,820	3,751
6	SaveAll in	1,600	36	12,790	2,790	9,264	7,830	5,620	3,300
3	SaveAll out	1,600	36	8,020	3,010	4,031	3,439	2,930	978
2	Total out	2,150	31	12,460	2,220	7,401	6,828	6,600	1,240

Site D					
ID	Stream	TKN (mg/L)	NH_4-N (mg/L)	TKP (mg/L)	PO_4-P (mg/L)
25	Paunch - pre screen	266	18	167	99
4	Paunch - post screen	243	13	146	88
24	Paunch - solids	776	N/A	243	N/A
21	Cattle wash - pre auger	356	86	65	29
22	Cattle wash - post auger	129	87	18	9
23	Cattle wash - solids	1,922	N/A	475	N/A
10	Combined bins	2,076	180	164	89
11	Combined stick	492	215	114	34
12	Veal room	294	26	4	15
9	Slaughter floor/offal	154	5	20	3
7	Combined red - pre screen	353	38	39	16
6	SaveAll in	420	27	41	19
3	SaveAll out	402	38	41	33
2	Total out	438	38	56	27

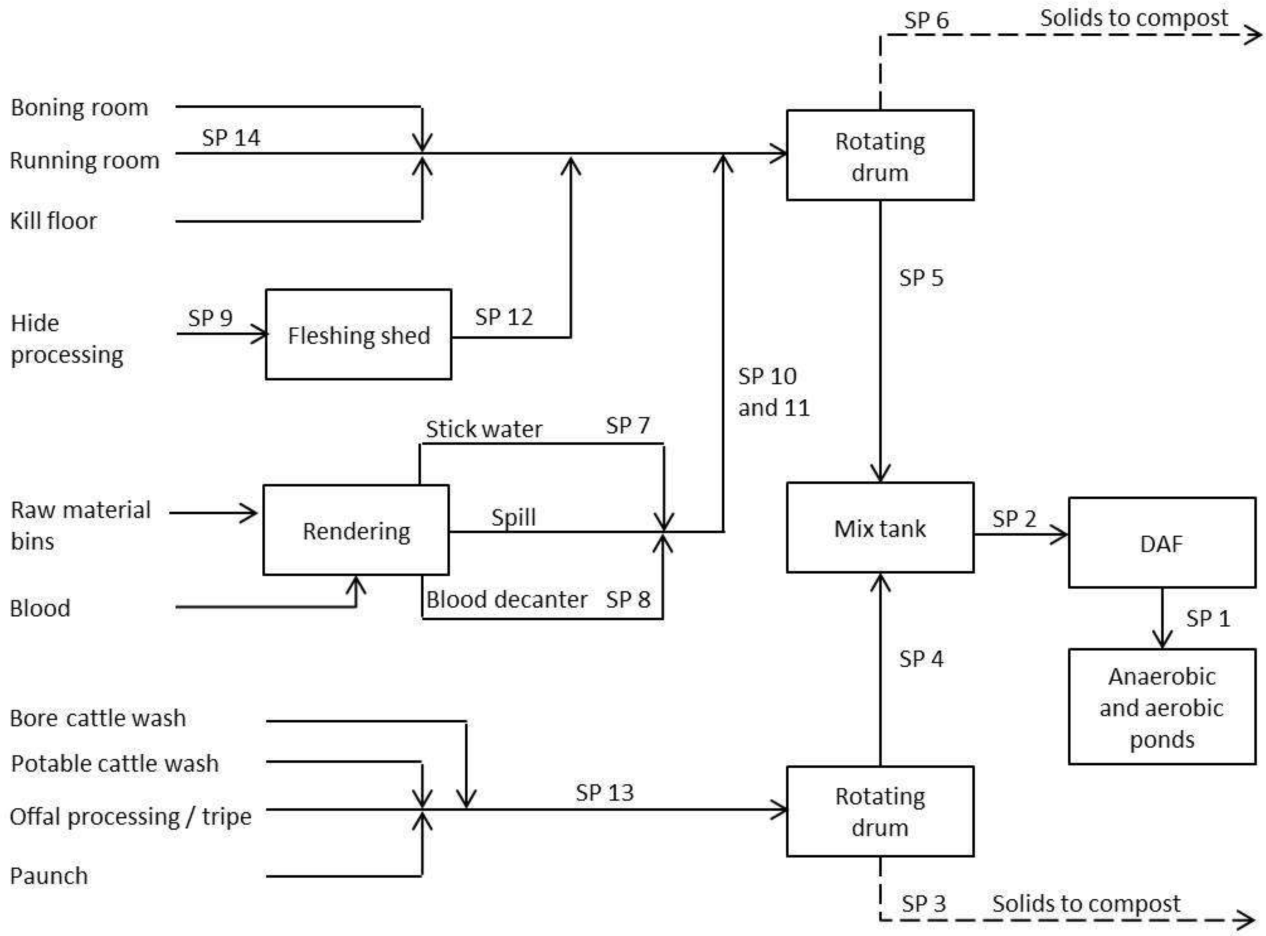


Figure A1.5. Flowsheet of wastewater sources, handling and treatment at Site E (adapted from Jensen and Batstone, 2013). SP: sample point.

9.1.6 Processing Site F

Site F is a small, family owned, Australian livestock processing facility situated in North Queensland. Site F processes cattle, veal, pigs and goats. Processing volumes and species vary through a typical week. Figure A1.6 and Table A1.6 show the flowsheet and wastewater characterization at Site F. As a very small processing facility, the waste and wastewater handling operations at Site F did not follow the same structure observed at other sites. In particular:

- Blood streams do not pass through the rendering plant.
- Paunch solids and blood streams do not pass through the wastewater treatment train and are handled using direct land application.
- Rendering wastewater is treated using a DAF designed to remove solids and recover FOG. This primary treatment is done on the rendering effluent only.

Table A1.5. Characterization of wastewater streams at Site E. SP: sample point (refer to Figure A1.5).

Site E												
ID	Stream	Volume (kL/d)	Temp (°C)	TCOD (mg/L)	SCOD (mg/L)	TS (mg/L)	VS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TP (mg/L)	PO_4-P (mg/L)
1	Total effluent	962	45	10,925	1,195	6,118	4,920	1,569	272	25	47	32
2	DAF in	953	46	12,214	1,247	6,678	5,745	2,380	292	22	42	32
4	Paunch liquid	104	33	11,788	778	8,152	6,081	900	319	56	108	44
5	Red post screen	953	49	9,823	1,548	5,380	4,569	1,985	248	10	24	21
7	Stick water	17	45	80,275	7,365	40,730	37,398	17,350	1,315	74	184	47
8	Blood decanter	64	48	32,918	14,148	22,101	15,451	300	2,777	26	87	47
9	Hide	179	28	2,193	1,500	1,916	1,280	20	166	2	5	11
10	Spill 1	28	49	388	181	684	352	24	15	0	1	0
11	Spill 2		79	180,750	3,540	124,927	122,770	72,600	2,010	54	211	27
12	Fleshing shed	120	33	2,642	981	2,135	1,640	144	96	1	8	8
13	Paunch pre screen	119	28	18,596	1,140	18,366	14,901	990	333	96	142	3
14	Running room	32	38	10,613	3,342	7,324	5,896	366	485	31	72	25

Table A1.6. Characterization of wastewater streams at Site F. SP: sample point (refer to Figure A1.6).

Site F												
ID	Stream	Volume (kL/d)	Temp (°C)	TCOD (mg/L)	SCOD (mg/L)	TS (mg/L)	VS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TP (mg/L)	PO_4-P (mg/L)
1	Cattle wash	4	19	4,347	1,013	4,117	2,939	60	218	115	33	13
2	Total render	17	37	21,936	2,370	10,241	9,631	9,578	513	153	70	34
3	Paunch & KF after trommel	99	37	2,631	708	2,086	1,734	148	98	49	15	6
5	Total effluent	168	33	6,719	1,148	3,471	3,038	2,258	178	74	27	12
7	Paunch solids	3	29	121,030	N/A	118,765	103,036	2,094	2,790	N/A	983	N/A
8	Cattle blood	23	33	43,065	2,128	21,873	20,785	864	4,093	385	50	36
9	Pig blood	13	36	3,906	3,252	2,968	2,704	24	375	25	9	4

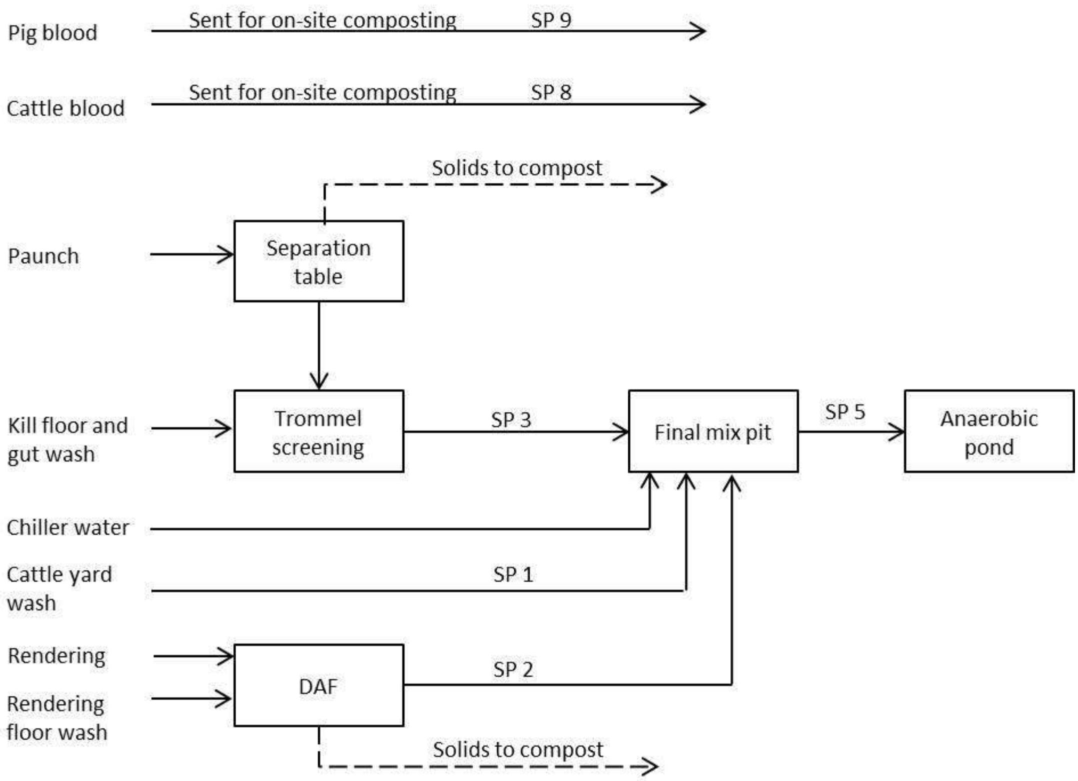


Figure A1.6. Flowsheet of wastewater sources, handling and treatment at Site F (adapted from Jensen and Batstone, 2012). SP: sample point.

9.1.7 Processing Site G

Site G has been approached by Murdoch personnel and agreed at providing data that can assist with the current project. Please note that the information provided by Site G is strictly confidential and for internal reference only. The operations at Site G include standard processes such as slaughtering and rendering and a flowsheet of the wastewater treatment process is shown in Figure A1.7.

The fresh water entering the meat processing plant varies seasonally at 50 – 60 ML per month during summer months, and 40 – 55 ML per month during winter. Non-potable water is supplied by the pipeline water supply scheme. The water is then chlorinated on site prior to storage in covered and locked tanks. The average consumption of water at Site G ranges from 2 to 5 kL per head per day. Note that no water reuse or recycle is undertaken. The total volume of the daily produced wastewater is unknown; however, the measuring system is going through several updates that will provide routine measurements of flow rate across the plant. The wastewater is directed from each process operation (i.e., slaughtering, boning and rendering, cleaning of chilling and freezing areas) into one drainage system through to the wastewater treatment plant. Solids are screened using a rotary wedge wire screen and sent for composting. The post-screening effluent is sent to a DAF-saveall unit to separate the remaining solids and allow floatation of fats. Solids are collected, de-watered and processed into compost. The effluent enters a settling pond where it is met with storm water from drainage. Solids are periodically removed from the settling pond and processed as soil conditioner. The primarily treated wastewater leaves the settling pond through a weir system and is pumped through to the anaerobic pond where anaerobic digestion occurs. A small percentage of the settling pond effluent, e.g. 10%, is added as raw feed into the aerobic pond. The effluent from the anaerobic pond is sent to an aerobic pond for biological nitrogen removal. The pond is composed of three sections: anoxic zone for denitrification, aerobic zone for nitrification and settling zone for sludge recycle. After the biological treatment, four storage ponds are used for storage over periods of high rainfall when irrigation is not required, as well as to provide further retention time for settling of solids, natural aeration and evaporation. Two biofilter plants are used to control odors from the rendering plant and to keep constant moisture ahead of the biological treatment. A fraction of the treated effluent is pumped to surrounding land for irrigation of pasture and perennial crops. The current wastewater treatment system does not incorporate phosphorous reduction; therefore, the abattoir removes phosphorous by the use of a cropping program. Selected crops are planted that have a high uptake of phosphorous, potassium and nitrogen then cropped and used as cattle feed.

Water and wastewater flow rates

There is only a limited amount of flow rate data available at Site G. The following information has been provided by personal communication with Site G personnel. Estimated flow rates are calculated based on the provided information and assumptions made by the authors.

The average consumption of water at Site G ranges from 2 to 5 kL/head/day (personal communication, 17 November 2016) and between 2,200 and 2,800 animals are processed weekly (personal communication, 17 November 2016). Assuming the plant operates 5 days a week, 440 to 560 animals are slaughtered every day, thus leading to a water consumption ranging from 880 to 2,800 kL/day. Our literature review has shown that similar values of fresh water consumption and wastewater generation are found in Australian abattoirs (Section 5.1 of the current report; Ridoutt et al., 2015). At Site G we assume that the amount of combined wastewater entering the treatment plant is within the same range as the fresh water consumed in the slaughtering process, i.e., from 880 to 2,800 kL/day. An average value of 1,750 kL/day of generated wastewater is considered in this project. If steady state is assumed in the operations of the wastewater treatment plant (i.e., wastewater coming in is the same as treated water going out on a daily basis), a flowrate equal to 1,750 kL/day is assumed throughout the wastewater treatment plant (i.e., at the monitoring stations ID 1, 2, 3, 4 and 5, Figure A1.7). The treated wastewater is withdrawn from storage ponds 3 and 6 (Figure A1.7) and used for irrigation. Table A1.7 shows the flow rate of the treated wastewater leaving the plant on a monthly basis from January to September 2016.

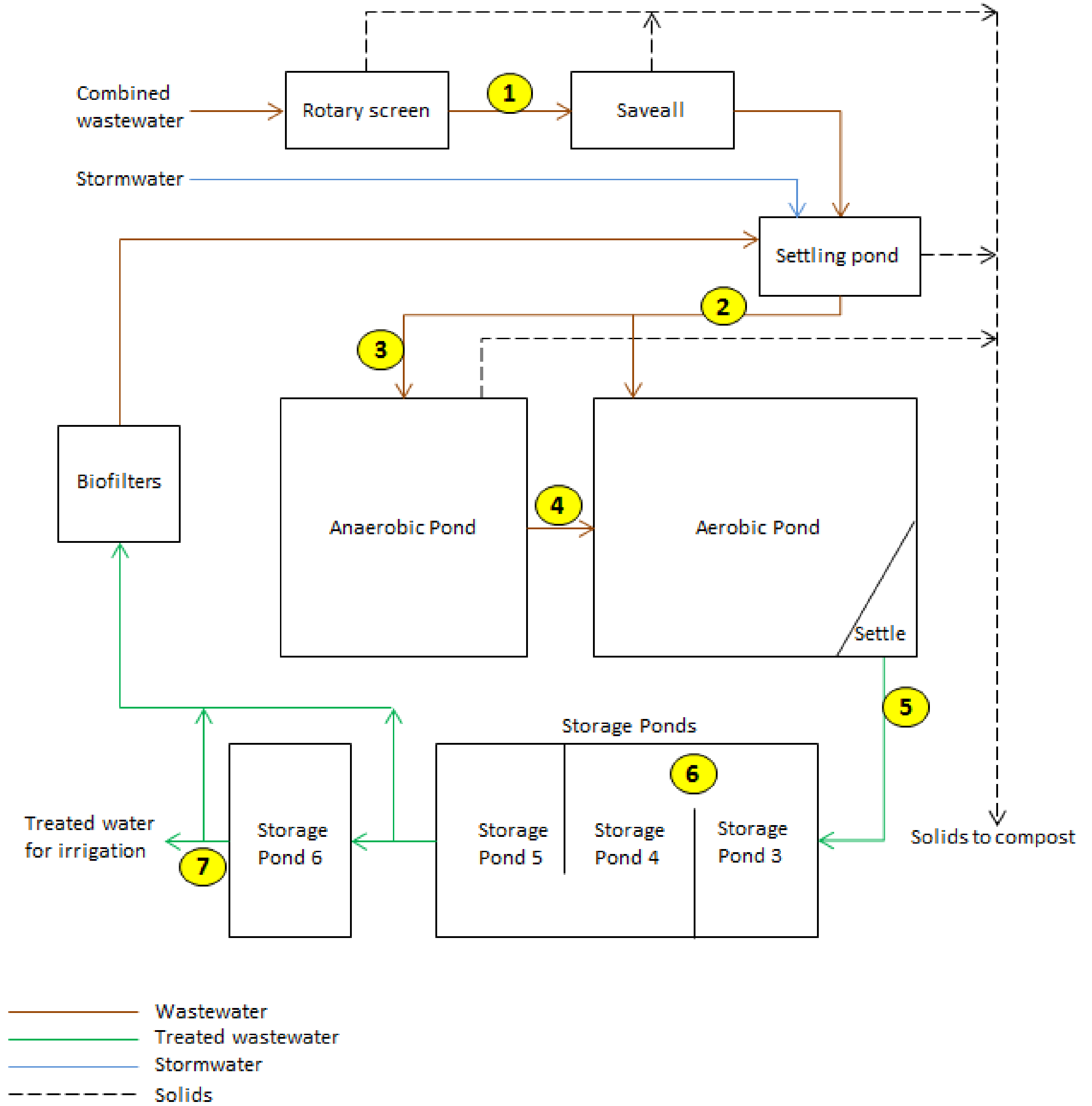


Figure A1.7. Flowsheet of the wastewater treatment plant at Site G. Water quality sampling points are highlighted.

Table A1.7. Flow rate of treated wastewater effluent used for irrigation at Site G.

Month	Flow from storage pond 3 (kL)	Flow from storage pond 6 (kL)	Total flow (kL)
January	455	816	1,270
February	317	953	1,270
March	249	796	1,045
April	485	916	1,401
May	474	1,053	1,526
June	729	964	1,693
July	379	823	1,202
August	370	1,146	1,515
September	379	984	1,363
October	NA	NA	NA
November	NA	NA	NA
December	NA	NA	NA

Water quality of wastewater streams

The quality of the wastewater is measured monthly at different sampling points distributed throughout the treatment plant. Site G has provided water quality data measured at the seven sampling points of Figure A1.7, at a monthly frequency and for three years (2014, 2015, 2016) for a total of 36 measurements at each sampling point.

Table A1.8 summarizes the average and standard deviation of nutrients and organic compounds calculated over the three-year period, from January 2014 to December 2016. Values of pH are not reported in the table but they have also been measured as a routine monitoring variable. A constant pH ranging from 6.6 to 6.8 has been measured at most sampling points. A lower pH (5.3 ± 1.2) is measured at the exit of the aerobic treatments (sampling point 5, Figure A1.7). Considerable variability with large standard deviations is found for FOG, BOD and TSS, while more consistent data are measured for nitrogen, phosphorus and TDS. Large differences of organic content in wastewaters are reported in the literature for different abattoirs; however, it is expected that similar concentrations are measured in time in the same abattoir. To better understand the variability in the data, each year has been analyzed separately, with particular attention on seasonal patterns. Tables A1.9, A1.10 and A1.11 summarize the average and standard deviation of the data calculated for each year separately. The year 2014 and 2015 show a large variability in FOG, TSS and BOD, whilst 2016 has more consistent measurements for BOD although large variability still exists for FOG and TSS. No obvious seasonal patterns have been detected in any water quality variable. It should be noted that the wastewater composition at the exit of the settling pond and at the entry of anaerobic pond (sampling points 2 and 3, Figure A1.7) should be the same. The data and their averages as reported in Table A1.8 are not overly different; however, some discrepancy occurs for the concentration of TDS, TSS and BOD. The raw data shows several missing values at sampling point 2 in 2014 and 2015, due to the water level in the pond being too low to take samples. A complete set of data is measured in 2016 and more consistent values are found at sampling point 2 and 3 during this year. In the following analysis, it was decided to ignore sampling point 2 and use the data measured at sampling point 3 as representative of the wastewater composition entering the AD process.

The efficiency of removal by each treatment process and by the overall wastewater treatment plant is calculated using the average values of each compound's concentration over the three years (Table A1.12) and over each year separately (Table A1.13). The efficiency of removal by the overall treatment is calculated as the difference between the compound's concentration measured at sampling point 1 (saveall entry, Figure A1.7) and the compound's concentration measured at sampling point 7 (settling pond 6 exit, Figure A1.7). The difference is then divided by the compound's concentration at sampling point 1 to express the removal as a percentage of the compound's concentration in the raw wastewater. The efficiency of removal by each treatment process is calculated as the difference between the compound's concentrations between two consecutive sampling points. Negative values indicate the concentration has increased between two consecutive sampling points. For example, negative values are expected for total nitrogen after anaerobic digestion as ammonium nitrogen is normally released by the AD process. Note that the efficiency of removal is calculated as a ratio of concentrations under the flow rate steady state assumption (same inflow and outflow at each treatment step). The wastewater treatment plant at Site G achieves high efficiencies of removal for FOG and BOD with 98% of the incoming concentration removed (Table A1.12). FOG and BOD concentrations are mostly lowered by primary treatments (saveall unit and settling pond) and by biological treatments. Only 37% of the nitrogen concentration entering the wastewater treatment plant is removed, despite the aerobic pond being specifically designed for nitrogen removal. The current wastewater treatment system does not incorporate phosphorous reduction, as seen by the TP removal efficiencies reported in Table A1.12 and Table A1.13. The removal of FOG and BOD has been consistent throughout the years with efficiencies higher than 95% (Table A1.13). Both nitrogen and phosphorous removal improved largely in 2016, with 62% and 32% of nitrogen and phosphorous removed, respectively (Table A1.13).

The water quality standards applicable to the treated wastewater effluent were given by Site G personnel and reported in Table A1.14. The load of each compound is estimated by multiplying the flow rates withdrawn monthly from storage ponds 3 and 6 (Table A1.7) with the compound's concentration measured monthly at sampling points 6 and 7 (exit of storage ponds 3 and 6, Figure A1.7). The load of each compound is calculated for the months from January to September 2016 as flow rate data haven't been provided from October to December 2016. Due to the absence of phosphorous removal, the load of total phosphorus always exceeds the guideline of 50 kg/year (Table A1.15). The total load of nitrogen is also above the guidelines despite having aerobic treatments operating at Site G (Table A1.15). Although the efficiency of nitrogen removal of the aerobic pond in 2016 was significantly higher than in the other years (Table A1.13), the nitrogen load in 2016 exceeds the limit of 300 kg/year (Table A1.15). The concentrations of FOG, BOD and TDS measured monthly at sampling points 6 and 7 in 2016 comply with the guidelines, as they are less than 50 mg/L for FOG and BOD and less than 1,500 mg/L for TDS. Unfortunately, no limits on nutrients' concentration have been communicated by Site G.

Table A1.8. Wastewater characterization at different sampling point for three years. Average and standard deviation are calculated on the complete dataset (from January 2014 to December 2016) at each sampling point.

Sampling point	ID	FOG (mg/L)			TN (mg/L)			TP (mg/L)		
Saveall entry	1	395	±	413	123	±	62	25	±	14
Settling pond exit	2	157	±	129	142	±	50	18	±	9
Anaerobic pond entry	3	179	±	219	122	±	35	19	±	7
Aerobic entry	4	8	±	5	151	±	42	26	±	7
Aerobic exit	5	5	±	1	126	±	37	27	±	6
Settling pond 3 exit	6	7	±	4	85	±	32	24	±	5
Settling pond 6 exit	7	7	±	5	78	±	30	24	±	6
Sampling point	**ID**	**TDS (mg/L)**			**TSS (mg/L)**			**BOD (mg/L)**		
Saveall entry	1	938	±	330	1,652	±	1,011	587	±	440
Settling pond exit	2	760	±	241	1,050	±	421	430	±	322
Anaerobic pond entry	3	658	±	198	1,169	±	1,049	664	±	1,040
Aerobic entry	4	672	±	98	530	±	770	54	±	54
Aerobic exit	5	757	±	154	457	±	933	14	±	15
Settling pond 3 exit	6	745	±	150	77	±	75	12	±	12
Settling pond 6 exit	7	755	±	168	70	±	64	12	±	12

Table A1.9. Wastewater characterization in 2014 at Site G. Average and standard deviation are calculated at each sampling point.

Sampling point	ID	FOG (mg/L)			TN (mg/L)			TP (mg/L)		
Saveall entry	1	494	±	383	83	±	32	22	±	13
Settling pond exit	2	250	±	108	136	±	89	9	±	3
Anaerobic pond entry	3	193	±	183	118	±	28	19	±	4
Aerobic entry	4	11	±	7	147	±	33	30	±	9
Aerobic exit	5	5	±	0	125	±	28	31	±	7
Settling pond 3 exit	6	7	±	4	82	±	34	27	±	5
Settling pond 6 exit	7	7	±	7	74	±	27	27	±	6
Sampling point	**ID**	**TDS (mg/L)**			**TSS (mg/L)**			**BOD (mg/L)**		
Saveall entry	1	754	±	294	1,319	±	860	630	±	642
Settling pond exit	2	630	±	210	920	±	515	388	±	372
Anaerobic pond entry	3	622	±	91	1,018	±	1,015	811	±	1,520
Aerobic entry	4	663	±	81	615	±	962	59	±	71
Aerobic exit	5	835	±	161	301	±	226	7	±	6
Settling pond 3 exit	6	793	±	138	121	±	113	8	±	9
Settling pond 6 exit	7	802	±	127	116	±	86	8	±	8

Table A1.10. Wastewater characterization in 2015 at Site G. Average and standard deviation are calculated at each sampling point.

Sampling point	ID	FOG (mg/L)			TN (mg/L)			TP (mg/L)		
Saveall entry	1	501	±	530	123	±	80	24	±	17
Settling pond exit	2	151	±	139	139	±	32	21	±	10
Anaerobic pond entry	3	202	±	262	117	±	36	20	±	9
Aerobic entry	4	8	±	5	158	±	63	27	±	6
Aerobic exit	5	6	±	2	132	±	19	25	±	3
Settling pond 3 exit	6	8	±	6	103	±	31	25	±	4
Settling pond 6 exit	7	7	±	5	98	±	30	25	±	4
Sampling point	**ID**	**TDS (mg/L)**			**TSS (mg/L)**			**BOD (mg/L)**		
Saveall entry	1	871	±	322	1,838	±	1,428	542	±	363
Settling pond exit	2	804	±	274	1,027	±	362	558	±	458
Anaerobic pond entry	3	637	±	288	1,038	±	876	863	±	956
Aerobic entry	4	721	±	84	548	±	918	55	±	38
Aerobic exit	5	742	±	132	242	±	118	25	±	21
Settling pond 3 exit	6	785	±	153	57	±	25	15	±	12
Settling pond 6 exit	7	818	±	184	45	±	29	13	±	11

Table A1.11. Wastewater characterization in 2016 at Site G. Average and standard deviation are calculated at each sampling point.

Sampling point	ID	FOG (mg/L)			TN (mg/L)			TP (mg/L)		
Saveall entry	1	191	±	213	163	±	37	30	±	11
Settling pond exit	2	120	±	122	146	±	41	20	±	8
Anaerobic pond entry	3	143	±	215	131	±	42	19	±	8
Aerobic entry	4	5	±	1	148	±	27	23	±	4
Aerobic exit	5	5	±	0	122	±	54	24	±	5
Settling pond 3 exit	6	5	±	0	70	±	25	21	±	5
Settling pond 6 exit	7	5	±	1	62	±	23	20	±	5
Sampling point	**ID**	**TDS (mg/L)**			**TSS (mg/L)**			**BOD (mg/L)**		
Saveall entry	1	1,189	±	214	1,798	±	540	586	±	247
Settling pond exit	2	788	±	233	1,124	±	435	373	±	194
Anaerobic pond entry	3	716	±	167	1,453	±	1,253	317	±	141
Aerobic entry	4	636	±	111	430	±	380	47	±	52
Aerobic exit	5	693	±	144	828	±	1,575	9	±	6
Settling pond 3 exit	6	662	±	132	57	±	48	14	±	14
Settling pond 6 exit	7	650	±	147	50	±	39	13	±	16

Table A1.12. Efficiency of removal by the wastewater treatment process calculated over the three years at Site G.

Treatment process	Efficiency of removal (%)					
	FOG	TN	TP	TDS	TSS	BOD
Saveall and settling pond	55	1	23	30	29	-13
Anaerobic treatment	96	-24	-36	-2	55	92
Aerobic treatment	32	17	-1	-13	14	74
Settling pond 3	-26	33	9	1	83	12
Settling point 6	3	8	-1	-1	9	6
Overall treatment	98	37	3	20	96	98

Table A1.13. Efficiency of removal by the wastewater treatment process calculated over each year separately at Site G.

Treatment process	Efficiency of removal (%)					
	FOG	TN	TP	TDS	TSS	BOD
			2014			
Saveall and settling pond	61	-42	14	18	23	-29
Anaerobic treatment	95	-24	-55	-7	40	93
Aerobic treatment	51	15	-4	-26	51	88
Settling pond 3	-33	34	12	5	60	-12
Settling point 6	-5	11	-2	-1	5	-2
Overall treatment	99	11	-24	-6	91	99
			2015			
Saveall and settling pond	60	5	15	27	44	-59
Anaerobic treatment	96	-36	-31	-13	47	94
Aerobic treatment	28	17	5	-3	56	55
Settling pond 3	-42	22	2	-6	77	41
Settling point 6	14	5	-1	-4	20	10
Overall treatment	99	20	-5	6	98	98
			2016			
Saveall and settling pond	25	20	37	40	19	46
Anaerobic treatment	96	-13	-21	11	70	85
Aerobic treatment	6	18	-3	-9	-92	80
Settling pond 3	0	43	11	5	93	-55
Settling point 6	-5	11	2	2	13	7
Overall treatment	97	62	32	45	97	98

Table A1.14. Water quality standards applicable to the treated wastewater effluent at Site G.

Parameter	Unit	FOG	BOD	TN	TP	TDS
Load	kg/year or kg/day	NA	< 30 kg/day	< 300 kg/year	< 50 kg/year	NA
Concentration	mg/L	50	50	NA	NA	1500

Table A1.15. Load of contaminants in treated wastewater effluent discharged for irrigation from January to September 2016 at Site G.

	FOG	TN	TP	TDS	TSS	BOD
Settling pond 3 (kg/year)	20	237	76	2,472	208	56
Settling pond 6(kg/year)	47	540	170	5,600	292	117
Total effluent (kg/year)	66	777	245	8,072	501	173
Annual load guideline at Site G (kg/year)	NA	< 300	< 50	NA	NA	NA

9.2 Appendix 2. Review of microalgae cultivation systems

9.2.1 Microalgae cultivation systems

The use of microalgae for the removal of organic contaminants and nutrients from wastewaters is referred to as phycoremediation (Benemann et al., 1977; Cuellar-Bermudez et al., 2017). Extensive research started to flourish in the eighties and more so in the last decade as microalgae cultivation systems have shown great potential in applications not only limited to wastewater treatment and nutrient reduction, but also for the production of biofuel, food supplements and pharmaceutical products (Oswald, 2003). In their pioneer work, Benemann et al. (1977) consider the huge potential of microalgae harvesting not only in wastewater treatment applications but also as a source of methane and fertilizer. Microalgae comprise a large group of autotrophic microorganisms with cells composed of proteins, carbohydrates, lipids, fatty acids, pigments, vitamins, and enzymes that can have value for human use (Cuellar-Bermudez et al., 2017). The production of biofuel is the most widespread application of microalgae cultivation as several advantages over traditional energy crops have been identified (Benemann, 2013; Cai et al., 2013). Microalgae have shown higher fuel yield potential and lower water demand than traditional energy crops, and have the ability to store significant amounts of energy-rich compounds which can be utilized for the production of several distinct biofuels including biodiesel and ethanol (Abou-Shanab et al., 2013). Microalgae have shown a rapid growth and a high oil content of 20 - 50% on a dry weight basis. Microalgae do not compete with crops for arable land and freshwater because they can be cultivated in brackish water and on non-arable land. Moreover, microalgae fix carbon dioxide very effectively as up to 50% of overall biomass is comprised of carbon, thus reducing greenhouse gas emissions and improving air quality (Cai et al., 2013). Algae biomass residue after lipid extraction can be used as a nitrogen source, such as a protein-rich animal feed or fertilizer for crops (Oswald, 2003; Benemann, 2013). To this end, the biochemical composition of biomass should be stable and suitable for the target use with a potential toxicity that meets the quality standard and a suitable market to consume the output of bioproducts (Zhang et al., 2016).

To date, the mass cultivation of algae for liquid biofuel production has been unsuccessful due to very low cost of fossil fuels, high environmental footprint of microalgae in terms of need for land, consumption of water and fertilizers (Park et al., 2011). A niche opportunity has been identified in the integration of algae cultivation processes with wastewater treatment (Craggs et al., 1997; de-Bashan et al., 2004; Park et al., 2011; Borowtizka and Moheimani, 2013; Jebali et al., 2015; Mennaa et al., 2015). Wastewater represents a continuous and abundant source of water and nutrients for algae biomass production, whilst algae cultivation represents an option to improve the treatment of wastewater by removing organic pollutants and reducing nutrient concentration. Coupling the treatment of wastewater with the cultivation of microalgae is therefore a win–win strategy for both pollution control and biofuel production (Craggs et al., 2013; Zhang et al., 2016): the risk of eutrophication due to effluent discharge is minimized by removing inorganic nutrients and microalgal biomass can be used for biofuels, animal feed, and fertilizer production. Several microalgae have been reported as good candidates for wastewater bioremediation including *Chlamydomonas* sp., *Euglena* sp., *Micractinium* sp., *Botryococcus* sp., *Coelastrum* sp., *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp. (Nwoba et al., 2016).

Algae cultivation systems have evolved in different technologies. The two most common cultivation system designs are known as open ponds and closed photobioreactors (Borowtizka and Moheimani, 2013; Zittelli et al., 2013); the first being commonly used for large-scale commercial production in favorable climatic conditions (Craggs et al., 1997; Raes et al., 2014). Although originally used only to hold wastes, open ponds were observed to reduce pollution such as organic matter and nutrient concentrations by allowing the growth of bacteria and microalgae (Benemann et al., 1977). Since then, they have been used as the main technology to harvest microalgae for wastewater treatment and pollution control (Oswald, 2003). The most accepted open pond cultivation systems are raceway ponds which are currently utilized in large scale commercial applications due to their low capital expenditure and simple operation. High rate algal ponds (HRAPs) are shallow, open raceway ponds and have been used for treatment of municipal, industrial and agricultural wastewaters since their large-scale production was proposed by Oswald and Golueke (1960). The algal biomass produced and harvested from these wastewater treatment systems can be converted through various pathways to biofuels, for example anaerobic digestion to biogas, transesterification of lipids to biodiesel, fermentation of carbohydrate to bioethanol and high temperature conversion to bio-crude oil (Park et al., 2011). The major operational differences between open ponds and closed photobioreactors are the number of factors that can be regulated and influenced to optimize and stimulate growth. In open systems, there is only a limited control over growth conditions (e.g., light), evaporation of water and invasion of non-desired species (Raes et al., 2014; Nwoba et al., 2016). Closed photobioreactors, on the other hand, are characterised by the better regulation and control of many of the important biotic and abiotic limiting growth factors. However, closed photobioreactors also have several disadvantages such as cooling requirement, which increases operational costs, and greater oxygen build-up, which reduces productivity (Raes et al., 2014).

Despite the promising outcome of many experimental tests, microalgae cultivation in wastewater still faces some scale-up challenges (Cuellar-Bermudez et al., 2017). There are many critical environmental (light and temperature), operational (pH, CO_2 and nutrients) and biological (zooplankton grazers and algal pathogens) parameters that affect microalgae cultivation in wastewater. In particular the complex and varying characteristics of the wastewaters, contamination of the algal culture by unwanted bacteria and competitive species, and unstable biomass production are the main issues that have traditionally limited the scale up of microalgal cultivation systems from experimental to pilot scale (Cai et al., 2013). The external contamination by other heterotrophic microorganisms is one of the main limitations of microalgae cultivation systems (Maroneze et al., 2014). *Chlorella* species are easily cultivated and efficient in the removal of nutrients; however, in large-scale applications contamination by other species has limited this species applicability (Taskan, 2016). The maintenance of a monoculture in full-scale is prohibitively expensive and technically difficult to operate (Maroneze et al., 2014). In this sense, improving microalgae culture stability is a challenge to be surmounted before the industrial application of microalgal heterotrophic bioreactors in wastewater treatment facilities. Latest research has moved from mono-cultures towards mixed microbial cultures as the latter have been shown more effective than pure cultures in biological treatment systems. The identification of the microbial species responsible for the treatment of pollutants is therefore important to improve treatment performance. Zhang et al. (2016) found that wild microalgal strains isolated from the local environment are particularly suitable for open-pond systems, especially for the cases when wastewater is used as a substrate for growth.

9.2.2 Current applications of microalgae cultivation systems in wastewater treatment

As a large producer of wastewater, the food industry has investigated the application of microalgae cultivation systems to enhance wastewater treatment. The majority of the existing literature use piggery wastewaters as a substrate to grow microalgae to *i)* improve the treatment of wastewaters and meet discharge criteria, and *ii)* produce biofuel from algal biomass.

One of the first applications of microalgae treatment on pig slurry is the pilot plant scale system tested by Fallowfield and Garrett (1985). A culture of *Chlorella* was used to treat piggery wastewaters. The wastewater went through some pre-treatments to remove solids and suspended matter in order to reduce light attenuation. A 9-time dilution was also required to further reduce turbidity. The authors found light as a limiting factor to be considered when treating piggery wastewaters with algae, however the removal of nutrients and organic matter was quite substantial. Phosphorus and nitrogen removal ranged from 42 to 89% and from 54 to 98%, respectively. BOD removal was approximately 98%.

A pilot plant was proposed and tested by Garden (2005) to improve ammoniacal-nitrogen treatment of wastewater and harvest algal biomass for biofuel production. The author identified induced air flotation as an appropriate algae separation technology and cultivated algae in a high-rate open pond. The harvesting of algae was successful and nitrogen levels were kept at very low concentrations. A colony of *Micractinium* sp. and *Scenedesmus* sp. were identified.

Zhu et al. (2013) tested an integrated approach which combined freshwater microalgae *Chlorella zofingiensis* cultivation with piggery wastewater treatment for biodiesel production. Piggery wastewaters were pre-treated by autoclave sterilization, to prevent contamination of the microalgae culture, and diluted with distilled water to five different COD concentrations. Pollutants in piggery wastewater were efficiently removed with COD, TN and TP removal ranging from 66% to 80%, from 69% to 83% and from 85% to 100%, respectively. The diluted piggery wastewater with an initial concentration of COD equal to 1,900 mg/L provided an optimal nutrient concentration for *C. zofingiensis* cultivation, thus resulting in the highest nutrient removal and productivities of biomass, lipid and biodiesel.

Six different microalgal cultures were cultivated in an experimental study by Abou-Shanab et al. (2013). The study aimed at improving wastewater treatment by reducing nutrient concentrations and producing biodiesel. Piggery wastewater effluents, that were biologically-treated by an anaerobic/oxic process, were used as a substrate for algal growth. The highest removal of nitrogen (62%), phosphorus (28%), and inorganic carbon (29%) was achieved by *Chlamydomonas Mexicana*, a microalgae belonging to Chlorophytes phyla. The authors suggest that *C. mexicana* is one of the most promising candidates for simultaneous nutrient removal and high-efficient biodiesel production.

To improve the cost-effective production of energy from algae cultivation systems, one promising application is the use of microalgae biomass in anaerobic digestion processes for the production of methane. Anaerobic digestion (AD) is a mature technology which uses microorganisms to decompose organic waste and produce biogas. Many AD systems have been built in European countries and the US for municipal, industrial, and agricultural waste treatment, and are recommended for COD concentrations higher than 4,000 mg/L (Cuellar-Bermudez et al., 2017). AD is a straightforward technology for microalgae biomass valorization and is preferred over biodiesel or bioethanol production since it avoids the drying step of the biomass and the three macromolecules (namely carbohydrates, proteins and lipids) are all converted to biogas (Molinuevo-Salces et al., 2016). The integration of the AD process and microalgae cultivation as a means to improve discharge effluent quality and methane yield has been investigated by the food industry. A variety of experimental studies has investigated the use of swine manure as a substrate to achieve high algal growth and then use the algal biomass as substrate for methane production. Molinuevo-Salces et al. (2016) tested the efficiency of a microalgae consortium to treat swine slurry at different temperatures, illumination periods and initial nitrogen concentrations (80 and 250 mg/L as ammonium). The swine manure was filtered and diluted approximately 7 and 22 times to achieve the desired ammonium concentrations of around 250 and 80 mg/L N-NH_4, respectively. Three microalgae species, i.e. *Chlorella vulgaris*, *Scenedesmus obliquus* and *Chlamydomonas reindhardtii*, were chosen based on their robustness to grow in wastewater. Favorable culture conditions (23 °C and 14 h of illumination) and high ammonium loads resulted in higher biomass production and greater nutrients removal rates. Methane yields in the range of 106-146 and 171 ml CH_4 g COD^{-1} were obtained for the biomasses grown in batch and semi-continuous mode, respectively. Hernández et al. (2013) tested a culture of *Chlorella sorokiniana* and aerobic bacteria on pig manure to produce second generation biofuels. Temperature and illumination were at 24 °C and 6000 lux for 12 hours per day. AD results showed that methane yield was highly influenced by substrate/inoculum ratio and by lipids concentration of the biomass. A maximum methane yield of 518 ml CH_4 g COD^{-1} was obtained.

Efforts on AD have been focused on the optimization of biogas yield and degradation of the volatile solids; however, the management and post-treatment of the AD effluent has largely been overlooked (Cai et al., 2013). AD effluents are typically low in carbon and high in ammonium nitrogen and phosphorus. Most of the AD effluent is separated by a dewatering system into liquid and solid fractions. The solid portion is usually composted then marketed as potting media or soil amendment, while the liquid portion is traditionally used as fertilizer for land application (Cai et al., 2013). Due to stringent regulations for discharge and the high nutrient concentrations typically found in AD effluents, efficient and cost-effective nutrient recovery methods should be considered in order to reduce the risk of nitrogen and phosphorus pollution from AD. The use of microalgae cultivation to treat AD effluents has started to be considered as a cost-effective solution that can decrease the nutrient content of the AD effluent thus meeting strict discharge criteria as well as enhance the production of methane by recycling the growing algae biomass back to the AD. Dilution of AD effluent is usually needed before feeding to microalgae cultivation systems in order to avoid the potential inhibition of algal growth due to high ammonium concentration and turbidity (Cai et al., 2013). In addition, as there is a significant amount of bacteria in AD effluent, proper pretreatments, such as filtration and autoclave, may be necessary to prevent the contamination of algae production systems (Cai et al., 2013).

Nutrient recovery from AD piggery effluent by microalgae has gained renewed interest over the last decade (Nwoba et al., 2016, and reference therein). Among the microalgae species, *Chlorella* sp. and *Scenedesmus* sp. appear to be the most robust and versatile due to tolerance to different wastewater conditions (Nwoba et al., 2016). The R&D Centre at Murdoch University has a strong capability in the application of microalgae cultivation systems to AD effluents. Ayre (2013) successfully harvested *Chlorella* species in a mixed algae culture with *Scenedesmus* species and diatoms. The mixed strain algal culture grew on undiluted and untreated AD piggery effluents sourced from an Australian piggery. The operations of raceway ponds were monitored over a course of 20 weeks with ammonia concentrations as high as 1,600 mg/L NH_3-N. The work by Ayre (2013) has shown that algal harvesting on piggery wastewaters is a promising application and the harvested biomass can potentially be used either as food source to enhance pig production or as a biomass to enrich the AD process. The author argues that, in order to sustain a quality controlled food source, growth of a single strain of algae might be the most attractive option for obtaining consistent protein, lipid and nutrient characteristics. Less control over the purity of the culture is required if algal biomass is to be used to enhance the production of methane by AD. Alternatively, algal biomass can also be used as a crop fertilizer. In all these scenarios the harvest of algae on AD effluents is seen as a positive impact on the operation costs and environmental footprint of Australian piggeries. Following the study by Ayre (2013), Nwoba et al. (2016) compared the growth of algae on piggery AD effluents in two different configurations, i.e. a paddle wheel raceway pond and a closed photobioreactor. Sand-filtered, undiluted AD piggery effluent was treated. While no significant differences were detected between the cultivation systems, the overall carbohydrate, lipid and protein contents of the consortium revealed its suitability to be used as animal feed or potential biofuel feedstock. The consortium was maintained in semi-continuous culture for more than three months without changes in the algal composition, thus indicating a promising outcome towards further testing and piloting of the proposed process. A recent study by Wang et al. (2016) demonstrated the successful growth of *Chlorella vulgaris* in a large scale application. Large-scale application was conducted in an open raceway pond with undiluted anaerobically treated piggery wastewater. The initial concentrations and removal rate of nitrogen and phosphorous were 421 mg/L TN and 89.5%, and 60 mg/L TP and 85.3%, respectively.

9.2.3 Application of microalgae cultivation in the meat processing industry

The adverse effects on the environment of abattoir wastewaters call for new processes capable of reducing the organic content and nutrient concentrations prior recycle and/or discharge of the treated effluent. Anaerobic (e.g., AD) and aerobic (e.g., nitrification to denitrification through activated sludge) treatments are commonly used in abattoirs; however, some major limitations exist. Although anaerobic treatments efficiently remove organic matters whilst producing biogas, nutrient removal is not effectively performed and high nitrogen and phosphorus concentrations are found in the effluent. Aerobic treatments are well known for efficiently removing organic matter as well as nutrients; however, their high energy requirement makes these methods expensive. Following the advantages of algal cultivation systems and their widespread use for wastewater treatment, researchers have recently started to investigate the integration of microalgae cultivation for the treatment of abattoir wastewaters.

Maroneze et al. (2014) studied the performance of microalgal heterotrophic bioreactors in the secondary treatment of cattle abattoir wastewater. The objective was to improve the effluent water quality and produce algal biomass for biodiesel production. The authors tested the alternative process of substituting conventional treatments (e.g., activated sludge and anaerobic systems) with microalgae-based systems in the secondary treatment of the wastewater. The wastewater composition was 7,692 ± 5193 mg/L COD, 155 ± 80 mg/L TKN and 23 ± 10 mg/L P-PO_4. No wastewater dilution was done before treatment by algae. *Phormidium*, which is a genus of single-cell blue green algae that belongs to cyanobacteria phylum, was grown in this experiment. The microalgal heterotrophic bioreactor converted in the order of 90% of COD, 57% of N-TKN, and 52% of

$P\text{-}PO_4$ in algal biomass. The nitrogen removal in the heterotrophic microalgal bioreactor was attributed to bioconversion of nitrogen into algal biomass as well as other non-biological processes, such as air stripping, ammonia volatilization, absorption, and sedimentation. Phosphorus removal was related to microalgal uptake, chemical precipitation and biosorption by microalgal biomass.

Taskan (2016) investigated organic matter and nutrient removal from abattoir wastewaters by a mixed microalgae culture (i.e. eukaryotic and cyanobacterial species) grown in a closed photo-bioreactor. The abattoir wastewater was filtrated with a microfiltration membrane to remove particles after it was sterilized by autoclaving. The main characteristics of the raw wastewater were 197 mg/L TOC, 102 mg/L TN, 18 mg/L TP. Pure as well as diluted wastewaters were used as substrate for algal cultivation. After 7 days of cultivation, the highest removal percentages of total organic carbon, total nitrogen, and total phosphorus were 90%, 70% and 96%, respectively. The dilution ratio was found to strongly impact on organic matter and nutrient removal performances. The highest amount of TOC removal was obtained from the pure wastewater confirming the high carbon removal capacity of mixed algal photo-bioreactors. TN removal was severely affected by its initial concentration and gradually increased with the increase of TN concentration in the wastewater. The highest nitrogen removal was achieved on undiluted wastewaters by biological assimilation. The removal of phosphorous was not affected by its concentration in the initial feed and was higher than 93% for all dilution ratios. The algal growth results indicated that the abattoir wastewater was a good source of nutrients and organic matter for the growth of algae. The high nutrient concentration of undiluted wastewaters has been shown as a potential source for algal biomass production associated with biodiesel production. The results indicated that cyanobacterial species were more efficient than eukaryotic species in removing nutrients.

Hernandez et al. (2016) studied the performance of two high rate algal ponds treating pig abattoir wastewaters. The objective was to produce biofuels (biodiesel, methane) from microalgae biomass. The wastewater was diluted 3 times using tap water to feed the ponds. The main characteristics of raw wastewater were 1,621 ± 81 mg/L COD, 9.2 ± 0.5 mg/L NH_4-N, 1.4 ± 0.1 mg/L TP. One pond was placed indoors under controlled conditions of temperature and light supply, while the other pond was placed in a greenhouse. The microalgae consortium was composed by *Chlamydomonas subcaudata*, *Anabaena* sp. and *Nitzschia* sp. High removal efficiencies were achieved in the high rate algal pond placed indoor (92%, 80% and 71%) and placed in the greenhouse (86%, 79% and 91%) for COD, ammonium and phosphorous, respectively. The authors identified that, despite the high biochemical methane potential, productivity is often low as a result of the strong microalgal cell walls that hinder the bacterial attack. A pre-treatment of microalgal biomass is therefore suggested as a way to enhance its biodegradability and increase methane production.

A summary of relevant publications on microalgae cultivation systems used in the wastewater treatment by the food and meat processing industry is given in Tables A2.1 and A2.2.

Table A2.1. Selected literature on the application of microalgae cultivation systems for the treatment of wastewaters generated by the food and processing industry. Primarily treated wastewaters are used as substrate for algal growth.

Reference	Wastewater source	Microalgae species	Wastewater characterisation as feed to microalgae (mg/L)			Performance (% removal)		
			COD	TN	TP	COD	TN	TP
Fallowfield and Garrett (1985)	Piggery raw wastewater – primarily treated and 9 times diluted	*Chlorella*		137.2	18.8	as BOD	54 - 98	42 - 89
Zhu et al. (2013)	Piggery raw wastewater – primarily treated and diluted	*Chlorella zofingiensis*	3,500 ± 63 (pre-dilution)	148 ± 4 (pre-dilution)	156 ± 8 (pre-dilution)	66 - 80	69 - 83	85 - 100
Maroneze et al. (2014)	Beef abattoir wastewater – primarily treated and no dilution	*Phormidium* species	7,692 ± 5,193	155 ± 80 as TKN	23 ± 10 as PO_4	90	57	52
Hernandez et al. (2016)	Pig abattoir wastewater – primarily treated and 3 times dilution	*Chlamydomonas subcaudata*, *Anabaena* species and *Nitzschia* species	1,621 ± 81 (pre-dilution)	149 ± 12 as TKN (pre-dilution)	1.4 ± 0.2 (pre-dilution)	86-92	79-80 (as NH_4)	71-91 (as PO_4)
Molinuevo-Salces et al. (2016)	Swine manure – filtered and 7 to 22 times diluted	*Chlorella vulgaris*, *Scenedesmus obliquus* and *Chlamydomonas reindhardtii*	3,750 ± 64 (pre-dilution)	1,762 ± 41 (pre-dilution) 80 – 250 (post-dilution) as NH_4	161 ± 2 (pre-dilution)		96-99 (as NH_4)	82 - 100
Taskan (2016)	Beef abattoir wastewater – primarily treated by sterilisation and filtration (no dilution)	Eukaryotic and cyanobacterial species	197 as TOC	102	18	90	70	96

Table A2.2. Selected literature on the application of microalgae cultivation systems for the treatment of wastewaters generated by the food and processing industry. Secondarily treated wastewaters are used as substrate for algal growth.

Reference	Wastewater source	Microalgae species	Wastewater characterisation as feed to microalgae (mg/L)			Performance (% removal)		
			COD	TN	TP	COD	TN	TP
Abou-Shanab et al. (2013)	Piggery wastewater effluents – biologically treated by anaerobic/oxic process	*Chlamydomonas Mexicana*	571 ± 8 as total carbon	56 ± 2	13 ± 0.6	29	62	28
Ayre (2013)	Piggery wastewater effluents – post AD, no dilution		220 (as TOC)	690 - 1,600 as NH_4	43			
Hernández et al. (2013)	Pig manure – primarily and biologically treated by nitrification denitrification	*Chlorella sorokiniana* and aerobic bacteria	616 ± 45	218 ± 20	50 ± 9	62	83 for NH_4	58
Wang et al. (2016)	Piggery wastewater effluents – post AD, no dilution	*Chlorella vulgaris*	745 ± 7	290 ± 3	24 ± 2	42 - 73	89	85

9.3 Appendix 3. Alternative water sources for integration with microalgae cultivation

9.3.1 Site G abattoir

Two streams have been identified at Site G that could be potentially integrated with the microalgae cultivation process.

The effluent of the anaerobic pond (Stream 4, Figure A3.1) is a good candidate for microalgae harvesting due to its low concentration of FOG and BOD and high nutrient content (Table A1.8). The concentration range of nitrogen and phosphorus is within the literature values (151 ± 42 mg/L and 26 ± 7 mg/L for TN and TP respectively) and the relatively low variability over time (low standard deviation) guarantees a consistent load of nutrients into the microalgae cultivation system. Moreover, given the low efficiency of removal of nitrogen by the existing aerobic treatment (Tables A1.12 and A1.13), the microalgae cultivation process could substitute the aerobic treatments currently used at Site G and improve the effluent water quality in terms of both nitrogen and phosphorus concentration and load. The reconfiguration of the wastewater streams is proposed in Figure A3.1.

The effluent of primary treatments (saveall and settling pond) could also be a good candidate for microalgae harvesting (Stream 3, Figure A3.2). The concentration of nutrients is similar to the post AD measurements and within the range of literature values suitable for microalgae growth. The concentration of BOD in Stream 3 is measured at 664 ± 1,040 mg/L (Table A1.8). By considering a BOD to COD ratio of 0.5 (Bazrafshan et al., 2012; Sunder and Satyanarayan, 2013) and the upper range of the BOD variability (large standard deviation of 1,040 mg/L), the concentration of COD in Stream 3 can be estimated in a range of 1,328 - 2,080 mg/L. Although this COD concentration might be too high for microalgae to grow, it is still within the range tested by Maroneze et al. (2014) and Hernandez et al. (2016). The concentration of FOG (179 ± 219 mg/L) could, however, limit the growth of microalgae on Stream 3 due to the growth of competitive bacteria. The reconfiguration of the wastewater streams is proposed in Figure A3.2. Experimental tests are recommended to verify the suitability of Stream 3 as a substrate for microalgae growth. If successful, the microalgae process could substitute the full biological treatment process of anaerobic and aerobic treatments.

Although a comprehensive long term dataset on water quality has been provided by Site G, wastewater flowrates are not available at the moment due to an outdated monitoring system. An estimate of wastewater flowrate of 1,750 kL/d has been used in this report to calculate the mass and energy balances of the proposed microalgae cultivation system (refer to Appendix 9.1 for details on how the wastewater flow rate was estimated).

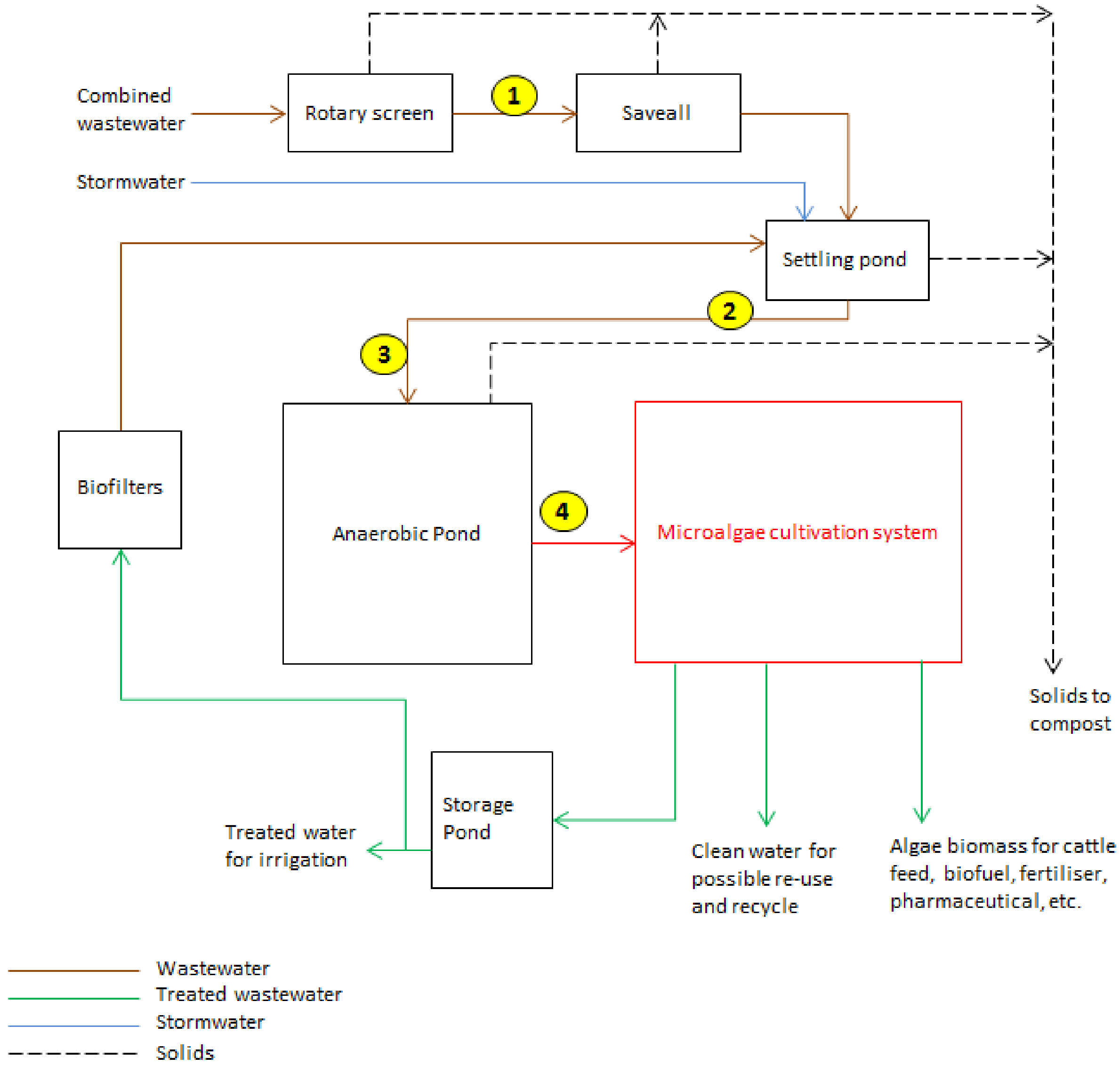


Figure A3.1. Flowsheet of the wastewater treatment plant at Site G which includes the proposed microalgae cultivation process (highlighted in red) applied on the AD effluent (Stream 4).

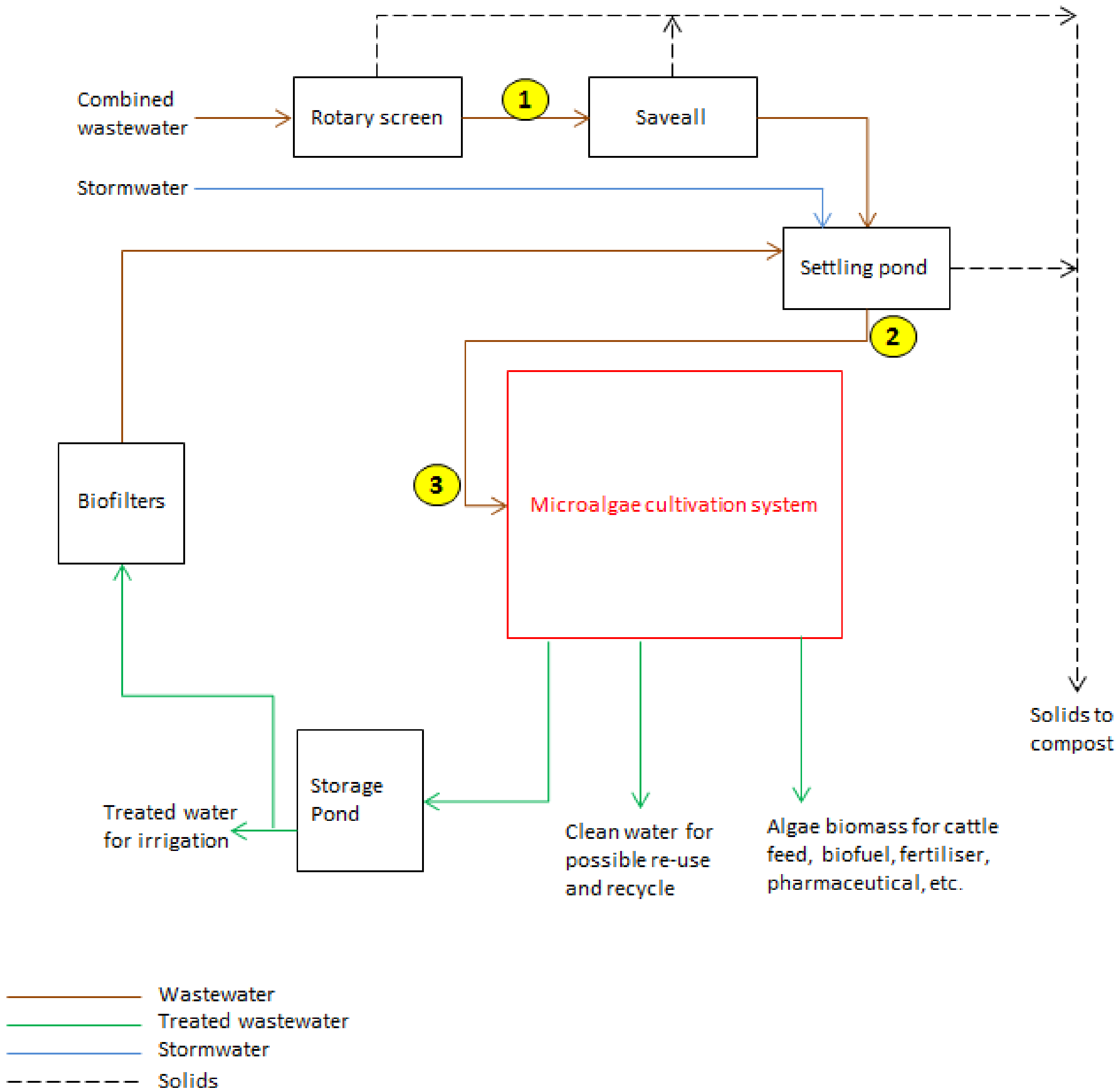


Figure A3.2. Flowsheet of the wastewater treatment plant at Site G which includes the proposed microalgae cultivation process (highlighted in red) applied on the primarily treated wastewater effluent (Stream 3).

9.3.2 Site A, C and D abattoirs

AD effluent characterization

Sites A, C and D follow a conventional treatment of their wastewaters which include screening and solid separation, dissolved air flotation units for fat and oil separation, and then combination of the wastewater streams in mixed tanks/open pits before entering anaerobic ponds/lagoons. Data on Sites A, C and D are sourced from the MLA/AMPC reports written by Jensen and Batstone (2012, 2013); however the AD effluent characterization was not given in those reports. Personal communication with one of the authors of the reviewed reports provided the composition of the AD effluent for Sites A and D. The nutrient concentration in the AD effluent at Site D is also reported in the MLA/AMPC report by Jensen (2015). AD effluent data are not available for Site C. The pre and post AD data at Sites A, C and D are summarized in Table A3.1.

An estimate of the AD effluent is calculated for Site C, as well as for the post AD FOG concentration at the three sites. Literature data on the efficiencies of removal of COD, BOD, FOG and nutrients by covered anaerobic lagoons are used as reference as these systems are currently operating at the selected Australian abattoirs. Mittal (2006) describes a typical low-rate anaerobic system (e.g., anaerobic lagoons and ponds) as a 3 to 5 m deep pond with a retention time of 5 to 10 days. Typical reductions of up to 97% BOD, 95% SS and 96% COD are reported in the literature (Mittal, 2006, and reference therein). In his MLA/AMPC report, Laginestra (2012) reviewed the efficiencies of covered anaerobic lagoons at a variety of Australian abattoirs. Despite a large variation in pond volume and hydraulic retention time, the COD removal varies from 80 to 88%, the BOD removal from 63 to 80% and the FOG removal from 83 to 95% (Laginestra, 2012). Typical effluent quality from anaerobic lagoons ranges from 500 to 1,500 mg/L BOD, depending on inflow and system optimization (Laginestra, 2012). No indication on nutrient removal is given in the report. COD removal efficiencies at Site A and D are calculated at 95% and 91%, respectively (Table A3.1), thus in line with literature values (Mittal, 2006). Based on literature data and removal efficiencies calculated at Sites A and D, a COD removal efficiency of 90% is assumed for Site C and a post AD COD concentration of 1,079 mg/L is estimated (Table A3.1, italics highlighted value). FOG removal by AD is considered equal to the average value given by Laginestra (2012) and equal to 89%. Post AD FOG concentrations are estimated for Sites A, C and D (Table A3.1).

The organic component of TKN and TKP normally accumulates in the AD pond (e.g., 40 to 50% accumulation of total phosphorous has been reported in anaerobic lagoons, Jensen, 2015); however, phosphorus as soluble PO_4 and nitrogen as NH_4 are found in the post AD effluent at high concentrations due to their release during anaerobic digestion processes. Removal of total nitrogen as low as 35% by AD is reported in the literature (Mittal, 2006). At Sites A and D, pre AD TKN is mostly formed by organic bound nitrogen and only a relatively small fraction is present as ammonia nitrogen (NH_4/TKN of 24% and 9% at Sites A and D, respectively, Table A3.1). On the contrary, post AD TKN is almost totally formed by ammonia-nitrogen as a consequence of its release during the biological degradation process (NH_4/TKN of 98% and 90% at Sites A and D, respectively, Table A3.1). As of phosphorous, at Site D, pre AD TKP is almost equally partitioned between organic bound phosphorus and phosphate, whilst post AD phosphorous is mostly formed by phosphate (Table A3.1). Mainly phosphate is measured at Site A both pre and post AD (Table A3.1). Overall, a large fraction of organic bound nitrogen and organic bound phosphorous accumulates in the anaerobic lagoon at Sites A and D, whilst high concentrations of ammonia and phosphate are found in post AD streams. The release of ammonia during the microbial digestion process at Sites A and D caused a 4 and 6 times increase of the post AD ammonia concentration, respectively (Table A3.1). Based on the post AD ammonia increase measured at Sites A and C, we assume a 5 times increase (average value between 4 and 6 as found at Sites A and D) in the concentration of ammonia post AD occurs at Site C. The concentration of ammonia in the post AD effluent at Site C is therefore estimated at 310 mg/L (Table A3.1). Due to the scarcity of data regarding phosphorus removal, we assumed that organic bound phosphorous at Site C is expected to accumulate in the AD process, whilst the same pre and post AD phosphate concentration is found at Site C (Table A3.1).

A reliable characterization of the post AD effluent is fundamental for a meaningful mass and energy balances of the microalgae cultivation system. Due to the uncertainty of our assumptions and the absence of measured nutrient values on the AD effluent at Site C, major focus is given on Sites A and D, for which a more reliable dataset is available.

Table A3.1. Wastewater characterization pre and post AD at Sites A, C and D. Data are taken from Jensen and Batstone 2012, Jensen and Batstone 2013, Jensen 2015. NA: not available data. Italics highlighted values are estimated based on literature and measured data.

	Site A		**Site C**		**Site D**	
Combined wastewater	Pre AD	Post AD	Pre AD	Post AD	Pre AD	Post AD
Volume (kL/d)	2,423	NA	2,115	NA	2,150	NA
TCOD (mg/L)	12,893	700	10,785	*1,079*	12,460	1,100
TS (mg/L)	8,396	NA	7,530	NA	7,401	NA
FOG (mg/L)	2,332	*257*	3,350	*369*	1,240	*136*
TKN (mg/L)	245	245	260	NA	438	254
NH_4-N (mg/L)	58	239	62	*310*	38	229
TKP (mg/L)	58	38	30	NA	56	34
PO_4-P (mg/L)	53	33	15	*15*	27	32

Selected wastewater streams

Although the nitrogen and phosphorous concentrations measured at Sites A and D are within the range measured at Site G and literature values, the concentrations of FOG and COD measured in the combined wastewater prior to AD are very high, thus making the pre AD stream not suitable for integration with a microalgae harvesting process (Tables A1.1 and A1.4).

Amongst the wastewater streams generated within the slaughtering process (Tables A1.1 and A1.4, Site A and D, respectively), some of them are characterized by a low organic and high nutrient content, thus making them suitable to be treated by microalgae. In particular:

- At Site A, the wastewater streams generated from cattle wash (SP 1, Table A1.1) and from the kill floor (SP 4, Table A1.1) could be combined and sent to a microalgae cultivation system. The composition of the resulting combined stream is reported in Table A3.2. The calculated COD concentration is 3,384 mg/L which is within the range of COD tested by Maroneze et al. (2014) and Hernandez et al. (2016). Following the integration with a microalgae cultivation process, the proposed reconfiguration of the process flowsheet is shown in Figure A3.3. Following the adoption of a microalgae cultivation system, the composition of Stream SP 8 would change and be the same as Stream SP 2 (Table A1.1). A proper mass balance is calculated in the next section to determine the final wastewater composition entering the microalgae cultivation system.
- At Site D, the wastewater streams generated from cattle wash (SP 22, Table A1.4) and from the

kill floor (SP 9, Table A1.4) could be used as substrate for microalgae harvesting. The composition of the resulting combined stream is reported in Table A3.2. The concentration of FOG might be too high (213 mg/L) for microalgae growth. If this is the case, Stream SP 9 generated by washing down the kill floor needs to be excluded from the microalgae treatment. The proposed reconfiguration of the process flowsheet is shown in Figure A3.4. A proper mass balance is calculated in the next section to determine the final wastewater composition entering the microalgae cultivation system.

Table A3.2. Characterization of the proposed combined streams for the integration with the microalgae cultivation process at Sites A and D.

Abattoir	Stream	Volume (kL/d)	TCOD (mg/L)	TS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TKP (mg/L)	PO_4-P (mg/L)
Site A	Combined streams SP 1 + SP 4	1,332	3,384	3,169	72	742	37	18	10
Site D	Combined streams SP 9 + SP 22	1,122	2,064	2,398	213	145	34	19	5

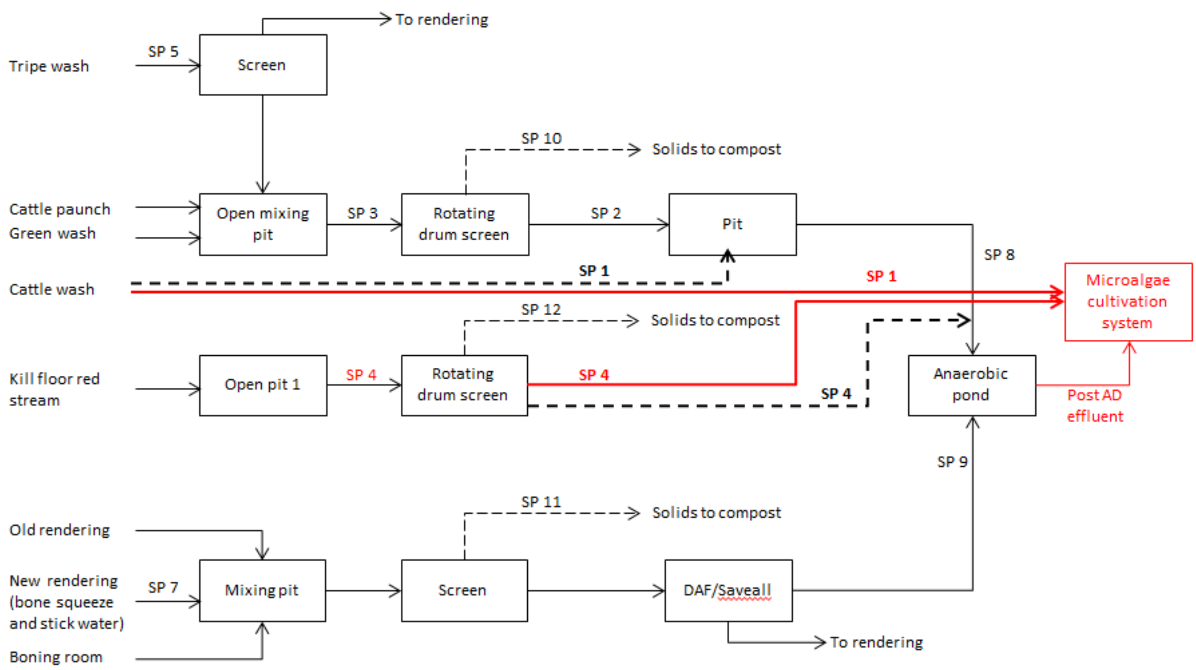


Figure A3.3. Reconfiguration of the process flowsheet at Site A. Black dotted lines: current flowsheet. Red lines: proposed reconfiguration by directing streams SP1 and SP4 to microalgae cultivation. SP: sample point.

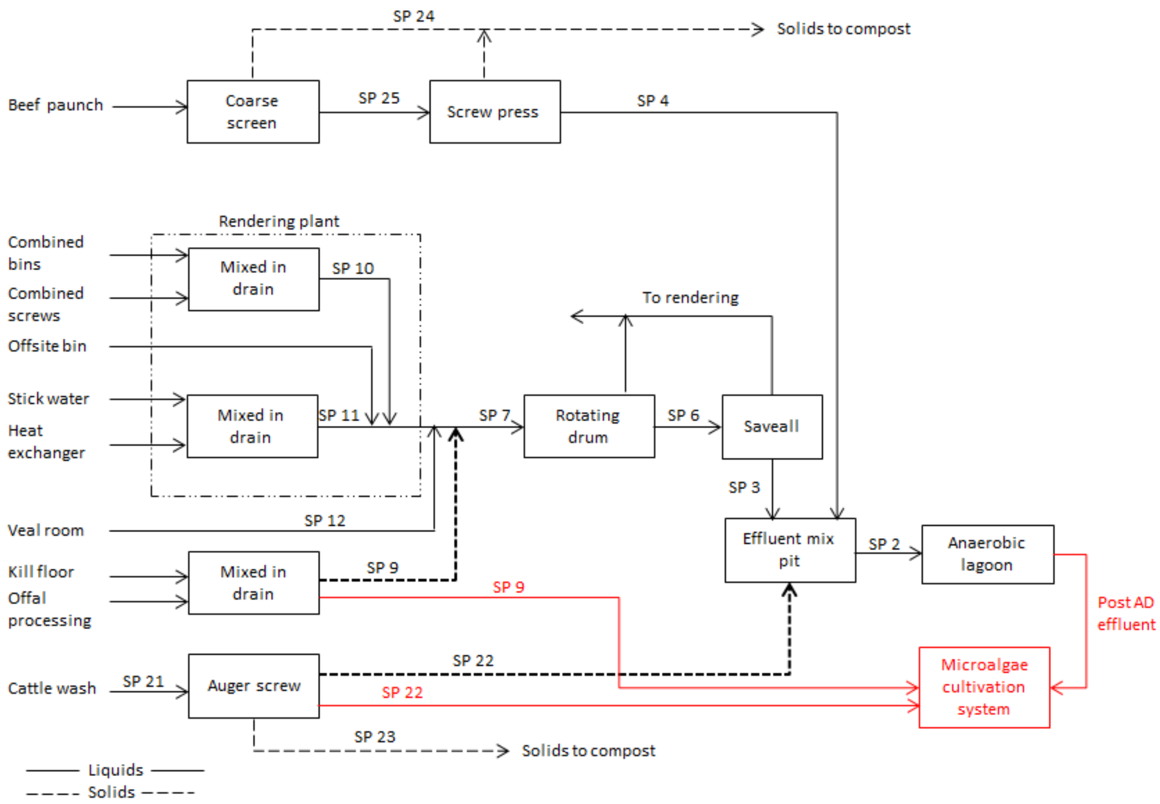


Figure A3.4. Reconfiguration of the process flowsheet at Site D. Black dotted lines: current flowsheet. Red lines: proposed reconfiguration by directing streams SP9 and SP22 to microalgae cultivation. SP: sample point.

Mass balance on selected wastewater streams

Although the AD effluent is considered the most suitable stream to grow microalgae on due to the low concentration of organics (COD and FOG) and high concentrations of ammonia and phosphorus, alternative wastewater streams generated by washing of cattle and kill floor have been identified at Sites A and D as potentially suitable for integration with the microalgae cultivation process. The composition of kill floor and cattle wash streams show a relatively low concentration of COD and FOG, as well as an adequate concentration of ammonia and phosphorus to grow algae (Table A1.1 and A1.4). The integration of kill floor and cattle wash wastewaters within the microalgae cultivation process considerably impacts on the process currently in place at Sites A and D, and requires a reconfiguration of the current flowsheet. By considering Site D as an example, the integration of kill floor (stream number SP9) and cattle wash (stream number SP22) wastewaters within the microalgae cultivation process causes changes in the current flowsheet as shown in Figure A3.4. Stream SP9 will by-pass preliminary treatments and both SP9 and SP22 will by-pass AD to be directly sent to microalgae cultivation (red lines, Figure A3.4). Such a reconfiguration of the process flowsheet will inevitably impact on the quantity and quality of the wastewater entering AD, therefore a mass balance on the impacted streams needs to be calculated.

The quantity and quality of each process stream refer to Table A1.4. Daily mass fluxes are calculated based on the volume of each stream (in kL/d) and each component concentration. Daily fluxes are reported in Table A3.3. The removal of SP9 from its current process streamline will affect the composition of SP7, SP3, and SP2. Similarly, the removal of SP22 will affect the composition of SP2. The composition of the affected streams (SP7, SP3, SP2, and AD effluent, as well as SP9, SP22, combined stream to microalgae) is summarized in Table A3.4. Note that the efficiency of removal of COD, FOG and nutrients by preliminary treatments (i.e., rotating drum and saveall) and by the AD have been calculated based on the removal rates as per mass balance (Table A3.3). Following the reconfiguration as proposed in Figure A3.4, the composition of the wastewater entering the AD process changes significantly. By comparing the composition of SP2 in Table A3.4 with its original composition in Table A1.4, it should be noted that *i)* the volume of water entering AD has halved (from 2,150 to 1,078 kL/d), *ii)* the concentration of COD increased from 12,460 mg/L to 21,267 mg/L, and *iii)* both TKN and TKP increased significantly. These changes are a consequence of the fact that a significant volume of wastewater (about 1,100 kL/d, sum of SP9 and SP22) with lower COD concentrations has been removed from the mix of streams entering AD, thus leading to a more concentrated pre AD stream. The post AD stream as calculated in Table A3.4 has COD and FOG concentrations that are potentially suitable to be treated in a microalgae cultivation system; however the ammonia and phosphate concentrations are almost double the post AD stream in the original case (compare AD effluent streams in Table A1.4 and Table A3.4).

It has to be noted that the COD concentration in the three streams (AD effluent, kill floor + cattle wash, combined post AD + kill floor + cattle wash) is about 1,900 mg/L (Table A3.4). Such a concentration of COD might be too high for a pure microalgae system as bacteria contamination could occur due to the high concentration of organics. This aspect will need to be assessed by specific bioprospecting experimental studies where a particular consortium of microalgae that is able to grow on the selected streams is identified.

The reconfiguration of the wastewater streams as proposed in Figure A3.4 and Table A3.4 has a considerable impact on the existing AD process due to the lower volume and higher strength of the new stream entering AD. The proposed solution does not seem to be optimal for the existing Site D abattoir and the integration of the microalgae cultivation system on the AD effluent only is considered a more practical solution for Site D. These calculations, however, suggest that, whether a new wastewater treatment plant is to be designed and a microalgae system considered, it is recommended to look at alternative streams within the slaughtering process to be integrated with the microalgae cultivation system. Both options of blending all streams together or design a dedicated microalgae cultivation system for each selected stream should be assessed during feasibility studies.

Similar results are obtained on Site A (Tables A3.5 and A3.6).

Table A3.3. Daily mass fluxes of each component in the wastewater streams at Site D. SP: sample point (refer to process flowsheet, Figure A3.4).

ID	Stream	Volume (kL/d)	TCOD (kg/d)	TS (kg/d)	FOG (kg/d)	TKN (kg/d)	NH_4-N (kg/d)	TKP (kg/d)	PO_4-P (kg/d)
SP25	Paunch - pre screen	200	2,438	3,025	28	53	4	33	20
SP4	Paunch - post screen	200	1,084	1,389	39	49	3	29	18
SP24	Paunch - solids	18	2,649	4,489	20	14	NA	4	NA
SP21	Cattle wash - pre auger	400	4,428	3,931	33	142	34	26	12
SP22	Cattle wash - post auger	400	720	792	4	52	35	7	4
SP23	Cattle wash - solids	2	179	312	1	4	NA	1	NA
SP10	Combined bins	304	13,419	9,287	2,826	631	55	50	27
SP11	Combined stick	94	6,901	3,152	1,981	46	20	11	3
SP12	Veal room	480	6,778	4,481	2	141	12	2	7
SP9	Kill floor/offal	722	1,596	1,899	235	111	4	14	2
SP7	Combined red - pre screen	1,600	15,920	13,582	6,002	565	61	62	26
SP6	SaveAll in	1,600	20,464	14,822	5,280	672	43	66	30
SP3	SaveAll out	1,600	12,832	6,450	1,565	643	61	66	53
SP2	Combined wastewater prior to AD	2,150	26,789	15,912	2,666	942	82	120	58
	Post AD effluent	2,150	2,365	NA	292	546	492	73	69

Table A3.4. Calculated composition of wastewater streams affected by the reconfiguration of the process flowsheet after integration with microalgae cultivation system. Site D. SP: sample point (refer to process flowsheet, Figure A3.4).

		Volume (kL/d)	TCOD (mg/L)	TS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TKP (mg/L)	PO_4-P (mg/L)
SP7	Combined red - pre screen	878	30,863	19,270	5,478	932	100	71	43
SP3	SaveAll out	878	24,876	9,150	1,428	932	100	71	43
SP2	Combined wastewater prior to AD	1,078	21,267	8,741	1,199	804	83	85	51
	Post AD effluent	1,078	1,877	NA	132	466	503	52	61
SP9 + SP22	Kill floor + cattle wash	1,122	2,064	2,398	213	145	34	19	5
post AD effluent + SP9 + SP22	Combined stream to microalgae	2,200	1,973	NA	173	303	264	35	32

Table A3.5. Daily mass fluxes of each component in the wastewater streams at Site A. SP: sample point (refer to process flowsheet, Figure A3.3).

ID	Stream	Volume (kL/d)	TCOD (kg/d)	TS (kg/d)	FOG (kg/d)	TKN (kg/d)	NH_4-N (kg/d)	TKP (kg/d)	PO_4-P (kg/d)
SP 1	Cattle wash	882	2,817	2,646	4	78	41	11	5
SP 2	Paunch liquid	311	7,435	4,914	810	161	11	66	50
SP 3	Paunch, tripe, green wash	330	10,793	8,184	1,281	93	5	51	33
SP 4	Kill floor	450	1,690	1,575	93	909	8	13	8
SP 5	Tripe wash	54	1,668	1,075	628	15	0	4	2
SP 7	New Render	192	7,681	4,723	1,063	330	8	23	14
SP 8	Primary treated wastewater cold	1,512	26,909	17,416	5,033	384	110	127	123
SP 9	Primary treated wastewater hot	911	6,567	4,373	1,037	241	40	26	15
	Combined wastewater prior to AD	2,423	32,929	21,443	5,956	626	148	148	135

Table A3.6. Calculated composition of wastewater streams affected by the reconfiguration of the process flowsheet as proposed after integration with microalgae cultivation system. Site A. SP: sample point (refer to process flowsheet, Figure A3.3).

		Volume (kL/d)	TCOD (mg/L)	TS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TKP (mg/L)	PO_4-P (mg/L)
	Combined wastewater prior to AD	1,222	11,459	7,600	1,511	328	42	75	53
	Post AD effluent	1,222	622	NA	167	328	173	49	33
SP1 + SP4	Kill floor + cattle wash	1,332	3,384	3,169	72	742	37	18	10
post AD effluent + SP1 + SP4	Combined streams to microalgae	2,554	2,062	NA	117	544	102	33	21

9.4 Appendix 4. Environmental impact and risk assessment

9.4.1 Guidelines for recycling of wastewater effluents in abattoirs

During this project, quantitative water quality guidelines applicable to the recycle of treated wastewater effluents at local as well as Australia-wide scale abattoirs have been referred to. Table A4.1 summarizes the water quality guidelines that are applicable to the permitted on-site and off-site uses of treated wastewaters in abattoirs. The main source of this information is the AGWR (Tables 3.8 and 4.10, AGWR, 2006), the AQIS meat notice (AQIS, 2008) and some information given by Site G personnel on local limits Site G abattoir must comply with. Although there has been a common perception that any water reuse and recycling would be unacceptable to export meat establishments, it is also expected and recommended by AQIS that the Australian meat processing industry would decrease its water usage by an efficient recycle of the treated wastewater effluent (AQIS, 2008). AQIS is in ongoing contact with regulatory agencies in Australia's export meat markets regarding the use of recycled and reuse water in abattoirs, and has incorporated their positions into the draft meat notice (AQIS, 2008; Warnecke et al., 2008). Table A4.1 focuses on those on-site and off-site uses where the wastewater has to be treated to fit-for-purpose levels without necessarily achieving potable quality. It should be noted that the list of AQIS-approved processes where the use of recycled wastewater is permitted is expected to grow, following successful validation and implementation by abattoirs (Warnecke et al., 2008). In abattoir wastewaters, the main potential hazards are caused by pathogens, pharmaceutical, nutrients and ammonia concentrations (Table 2.3, AGWR, 2006). The following analysis will mostly focus on nitrogen and phosphorous content.

9.4.2 Conventional treatment process at Sites A, D and G

Sites A, D and G follow a conventional treatment of their wastewaters which include screening and solid separation, dissolved air flotation for fat, oil and grease separation, and then combination of the wastewater streams in mixed tanks before entering anaerobic ponds/lagoons. Methane is collected during the AD process at Sites A and D and used as an energy source within the abattoir operations to lower their carbon footprint. No methane collection occurs at Site G. The composition of the AD effluents at Sites A, D and G is reported in Table A4.2. The management and post-treatment of the AD effluents generated in abattoirs have largely been overlooked, despite their major environmental impacts due to the high volumes of nutrient-rich wastewaters produced on a daily basis (Cai et al., 2013). The high nutrient content prevents the discharge of AD effluents into surface water bodies (compare nutrient concentrations in Table A4.2 with guidelines in Table A4.1). The quality of the AD effluents generated in abattoirs would allow the discharge of AD effluents into sewer systems for further treatment in off-site wastewater treatment plants; however, the costs associated with conveyance and off-site treatment make this option unfeasible. Typically, the management of AD effluents generated in abattoirs involves the storage of high volumes of water in evaporation ponds. After some retention time in the ponds (normally > 25 days as per AGWR, 2006), part of the water is used for irrigation of fields and paddocks. The advantages of storage ponds is that they are cheap, robust and easy to maintain, they provide a buffer to dilute possible peaks in chemical and microbial hazards as well as reduce enteric pathogens (AGWR, 2006). The reduction of nutrient concentration is however very low and high evaporation rates cause an increase in nitrogen, phosphorous and salinity in the long term. Moreover, volatilization of nitrogen into ammonia gas can occur, thus increasing the carbon footprint of the abattoir due to potential emission of greenhouse gases.

Table A4.1. Water quality standards applicable to the recycle of treated wastewater effluent for on-site and off-site non-potable uses.

Permitted uses of recycled wastewater	Water quality target	Source
On-site Cleaning of yards, infrastructures and trucks Washing of animals (other than final wash) Animal drinking water (older than 12 months) Fire control Steam production Toilet flushing Irrigation of green areas	• Secondary treatment + disinfection and/or lagoon detention (> 25 days) • BOD < 20 mg/L • SS < 30 mg/L • E. Coli < 100 cfu/100mL • TN < 40 mg/L [1] • NH_4-N < 35 mg/L [1] • TP < 12 mg/L [1]	AGWR, 2006
Off-site Irrigation of crops for fodder production	• Secondary treatment + disinfection and/or lagoon detention (> 25 days) • BOD < 20 mg/L • SS < 30 mg/L • E. Coli < 100 cfu/100mL • TN < 40 mg/L [1] • NH_4-N < 35 mg/L [1] • TP < 12 mg/L [1] • BOD < 50 mg/L [2] • BOD < 30 kg/year/hectare [2] • TDS < 1,500 mg/L [2] • TN < 300 kg/year [2] • TP < 50 kg/year [2]	AGWR, 2006 Site G abattoir
Final discharge To sewer	• BOD: 300 – 3,000 mg/L • COD: 900 – 9,000 mg/L • TSS: 1,000 – 1,500 mg/L • TN: not specified • FOG: 50 - 200 mg/L	AGWR, 2006
To surface water	• BOD: 5 – 10 mg/L • COD: 15 – 30 mg/L • TSS: 10 – 15 mg/L • TN: 0.1 – 15 mg/L • FOG: 2 - 15 mg/L	AGWR, 2006

[1] Indicative value given in Table 4.10 by the AGWR (2006). Values are not site-specific and might not be applicable to the abattoirs discussed in this project.
[2] Refer to Site G only.

Table A4.2. Composition of the treated wastewater effluents at Sites A, D and G. NA: not available data. Italics highlighted values are estimated based on literature data and assumptions made by the authors.

	Volume (kL/d)	TCOD (mg/L)	TS (mg/L)	FOG (mg/L)	TKN (mg/L)	NH_4-N (mg/L)	TKP (mg/L)	PO_4-P (mg/L)
Site A – AD effluent	*2,423*	700	NA	*257*	245	239	38	33
Site D – AD effluent	*2,150*	1,100	NA	*136*	254	229	34	32
Site G – AD effluent	*1,750*	54 ± 54 as BOD	530 ± 770 as TSS	8 ± 5	151 ± 42 as TN	NA	26 ± 7 as TP	NA
Site G – storage pond effluent	45	12 ± 12 as BOD	70 ± 64 as TSS	7 ± 5	78 ± 30 as TN	NA	24 ± 5 as TP	NA
Site G – annual load from storage pond effluent used for irrigation		173 kg/year as BOD	501 kg/year as TSS		777 kg/year as TN		245 kg/year as TP	

The use of treated abattoir wastewater for irrigation is widely applied in abattoirs as it represents a low-cost approach of wastewater management and can act as a good source of nutrients for infertile soil (Matheyarasu et al., 2015). The impacts of the discharge of abattoir wastewaters into the environment (soil, air and water) through crop irrigation are broadly classified into health and social impacts, and ecological impacts. As for health and social impacts, possible pathogens contamination of the surface and groundwater sources and odor spread during irrigation of sites constitute the most relevant impacts associated with the use of abattoir wastewaters for irrigation. The ecological impacts related to the storage in open ponds of wastewater effluents and their use for crop irrigation include:

- Generation of large volumes of water with high nutrient concentrations, thus not suitable for on-site recycle, except for irrigation of gardens, green areas and trees;
- Possible greenhouse gas emissions due to storage in open ponds of large volumes of water with elevated content of ammonia nitrogen;
- Potential eutrophication of soils and surface waters, nutrient imbalance, pest and disease in plants due to the high content of nitrogen and phosphorous in the water used for irrigation (toxicity of phosphorous to some native plants, AGWR, 2006);
- Potential nitrogen contamination to groundwater;
- Inefficient use of large areas of land dedicated to evaporation and storage ponds;
- Potential uncontrolled growth of algae (e.g., algal blooms) with spread of potentially toxic algal species;
- Loss of the environmental and economic value of the treated wastewater effluent associated with potential resource recovery of water, nitrogen and phosphorus.

Due to the detrimental environmental impacts associated with the use of abattoir wastewaters for irrigation, it is crucial that the following steps are considered (Matheyarasu et al., 2015):

- The discharged wastewater should not exceed the acceptable level of nutrients and pollutants, both in terms of concentration and loading rates;
- Microbial community should be eradicated through disinfection;
- The environmental standards (legislation/law) defined by the state environmental authority should be strictly followed;
- Pollution levels are to be reduced through various treatment techniques to retain the environmental quality.

The concentration of the treated wastewaters after retention in storage ponds is not available for Sites A and D; however, data are available for Site G (Table A4.2). Based on the guidelines summarized in Table A4.1, the concentration of nutrients in the final effluent at Site G is too high for recycle in uses other than irrigation of gardens, green areas and trees. As a consequence, only a small portion of the water stored in the storage ponds (on average 45 kL/d, Table A4.2) is reused for irrigation, thus leaving the majority of the water in ponds. Even when used for irrigation, the annual loads at Site G often exceed the local limits on the nutrient loading rates (compare Table A4.1 and Table A4.2).

The environmental impact and risk assessment of the conventional treatment system, management and uses of the wastewater effluents currently implemented at Sites A, D and G is summarized in Table A4.4. Nitrogen and phosphorous are considered the main environmental hazards. Based on the hazards and current uses of the treated wastewater, general environmental endpoints identified for consideration are groundwater, surface water, and soil. Air might also be impacted by the possible volatilization of untreated ammonia in the AD effluents while stored in evaporation ponds. Based on the current uses and the guidelines on the nitrogen and phosphorous concentrations, the high nutrient content in the wastewater effluent at Sites A, D and G identify potential high risks to the environment, thus requiring preventive measures to reduce the risk. More thorough and further treatments of the AD effluents are considered the most, and only, effective measure to lower the risk of high nutrient contamination. The objective of further treatment is mainly to ensure nutrients level in the wastewater effluents comply with the guidelines given in the AGWR (2006) in order to allow recycling and reuse of the treated wastewater. Once the environmental impacts and associated risks are managed from moderate/high to low/acceptable, the residual risks related to the identified uses are minimized and the environmental impacts reduced to acceptable levels.

A series of alternative treatments of AD effluents aiming at reducing nitrogen concentrations have been reviewed in a MLA/AMPC report by Jensen et al. (2013). The suggested solutions involve technologies such as nitrification/denitrification by activated sludge, anaerobic ammonium removal by ANNAMOX, and constructed wetlands (Jensen et al., 2013). Each technology presents several limitations that have hindered their adoption on a large scale. Activated sludge has elevated energy demand, thus high costs associated with aeration. Anaerobic ammonium removal processes are limited by high COD to ammonium ratios and by large fractions of non-degradable COD, both characteristics typically found in AD effluents from abattoirs wastewaters (Jensen et al., 2013). Constructed wetlands are easy to operate; however, they have shown poor nutrient removal rates and prohibitively large footprint (the wastewater production expected from Australian slaughterhouses is in the range of 1,000-3,000 kL/d which could require a wetland trench length in the range of 50-100 km, Jensen et al., 2013). The study by Jensen et al. (2013) concludes that an optimal treatment process has not been uniquely defined yet and has to be determined on a case by case basis. Microalgae cultivation systems might find a competitive niche application in the treatment of AD effluents from abattoirs.

9.4.3 Microalgae cultivation system at Sites D and G

Based on the mass balance developed in Section 5.4, the integration of the microalgae cultivation system on the AD effluent at Sites D and G generates two product streams: a concentrated microalgae biomass product and a wastewater effluent stream that is depleted in nutrients. The estimated composition of each product stream at each abattoir is summarized in Table A4.3. A full characterization of the quality of the treated wastewater effluent post microalgae cultivation is not available at this stage, as only through pilot testing the exact determination of the final stream composition can be quantified.

Table A4.3. Composition of the treated wastewater effluents at Sites D and G when the microalgae cultivation system is integrated with the current treatment process flowsheet. NA: not available data.

	Volume (kL/d)	Algae biomass (g/L)	Algae biomass (kg/d)	NH_4-N (mg/L)	PO_4-P (mg/L)
Site D					
Algal biomass stream	17	300	5,221	NA	NA
Wastewater effluent	1,270	0.05	64	0	< 0. 5
Site G					
Algal biomass stream	9	300	2,802	NA	NA
Wastewater effluent	1,300	0.05	64	0 as TN	1.4 as TP

The environmental impact and risk assessment is summarized in Table A4.4. A qualitative characterization of the environmental risks is determined by following the procedure described in Chapter 4 and Table 2.7 of the AGWR (2006). The concentration and loads of nitrogen, phosphorous and the residual concentration of algae biomass in the wastewater effluent are considered the main potential hazards. The residual concentration of algae biomass potentially present in the wastewater effluent is due to the assumed 90% efficiency of the dewatering and harvesting system. The presence of algae biomass represents a potential hazard related to the reuse and recycle of the wastewater effluent; however, specific guidelines are not well defined in the AQIS Meat Notice (AQIS, 2008) or in the Australian Guidelines for Water Recycling (AGWR, 2006). A detailed evaluation of the potential toxicity associated with the presence of algae biomass in the wastewater effluent is required and further filtration before reuse of the wastewater effluent might be necessary.

A nutrient-depleted wastewater effluent of about 1,300 kL/d at Sites D and G is a high-value process output. Subjected to water recycling guidelines implemented at each abattoir, the nutrient-depleted wastewater effluent can be recycled within the abattoir operations for all the uses proposed in Table

A4.4. Treated wastewater recycling improves the environmental footprint of each abattoir by reducing quantity and costs associated with freshwater usage. If recycle is not viable, the nutrient-depleted wastewater effluent is likely to meet the guidelines for safe discharge into surface waters (Table A4.1). The environmental endpoints potentially impacted by the recycling of the wastewater effluent are soil, surface water, groundwater and the abattoir's wastewater treatment plant. Off-sites uses, such as discharge to surface water bodies and irrigation of pasture for fodder production, are expected to cause environmental impacts such as nutrient imbalance, eutrophication and contamination. However, due to the low nutrient content in the wastewater effluent treated by the AD-microalgae cultivation process, the risk associated with such environmental impacts is classified as 'low', thus no preventive measures are required. On-site uses of the wastewater effluent can potentially cause contamination of potable water if the reticulation system is not well designed, operated and maintained, thus raising the potential risks to 'high' (Table A4.4). Preventative measures that guarantee a complete separation of potable and recycling water distribution systems are required, together with proper personnel training and Hazard Analysis Critical Control Point (HACCP) procedures (Table A4.4). Whenever these preventative measures do not lower the risk to acceptable levels, a stricter regulation on the permitted on-site uses of recycled wastewater is required until the risk is classified as 'low'.

The final use of the concentrated microalgae biomass has to be evaluated on a case-by-case basis depending on the location and needs of the abattoir, local market for algae products, costs of handling and transportation of the biomass. Amongst its possible uses, on-site uses such as biogas production through recycle to AD, use as fertilizer for crop irrigation, and use as animal feed are some technically feasible and cost effective uses of the algae product (Table A4.4). Off-site uses such as pharmaceutical products and biofuel generation could also be possible depending on the local market and the grown algal strain (Table A4.4). The evaluation of the environmental impacts associated with off-site uses of the algae biomass requires a full life cycle assessment, which at this stage is considered of low benefit to the current high-level desktop study due to the scarcity of data on each abattoir and process uncertainty (bioprospecting experimental tests are needed to lower the process uncertainty). The environmental impacts associated with the on-site uses of the biomass are not associated with potential risks as they are expected to improve the environmental footprint of the abattoir. The use of microalgae biomass (in particular *Chlorella* species) as crops fertilizer has shown to lead to positive effects on the environment in terms of the health of soils and plants. Similarly, the use of microalgae as animal feed has shown great potential in recent studies (Benemann, 2013), with *Chlorella* being one of the most robust and versatile species in the market.

In summary, positive and negative environmental impacts have been found for the integrated AD-microalgae cultivation process. The main negative environmental impact is associated with the recycle of the wastewater effluent for on-site operations such as cleaning of yards, infrastructures and trucks, washing of animals, and animal drinking water. Possible contamination of the potable water mains due to poor maintenance, design and/or misuse of the recycled water reticulation can lead to high risks of contamination of meat products, thus making preventive measures and appropriate hazard managing procedures a priority to minimise the risks. Another negative environmental impact relates to the large land footprint dedicated to microalgae cultivation systems in open ponds (21 and 11 hectares have been calculated for Sites D and G, respectively).

Several positive environmental impacts of the microalgae cultivation process have been identified as follows:

- Reclamation of the environmental and economic value contained in the wastewater effluent in terms of water and nutrient recovery;
- Generation of large volumes of water that are suitable for recycling for on-site and off-site uses or safe to discharge to surface water bodies;
- Generation of an algae biomass product that is suitable for re-use within the abattoir's operations or for sale to available markets.
- Sequestration of carbon dioxide currently generated by abattoirs during the current wastewater treatment process and fixation into algae biomass.

A comparison between the conventional treatment process and the integrated microalgae cultivation system points out that, under conventional treatments, large volumes of wastewaters are mostly stored in open ponds. This practice leads to potentially high environmental risks as identified in the environmental impact assessment. The wastewater effluents often do not adhere to the guidelines for water recycling and significantly impact soil, surface water, groundwater and air. Moreover, conventional treatments currently adopted in abattoirs do not consider the intrinsic value of water and nutrients within the wastewater effluent, thus wasting resources potentially suitable for recovery. From an environmental perspective, further treatment of the wastewater effluent is therefore unavoidable.

The microalgae cultivation system mitigates, and possibly removes, the environmental impacts associated with contamination of soils, water and groundwater, and to greenhouse gas emissions into the air. More importantly, it gives value to the wastewater effluent by recovering the nutrients and water and ultimately improving the environmental footprint of the abattoir. The main source of negative environmental impacts of the integrated microalgae cultivation process is related to possible misuse of the recycled water within the abattoir operations in those operations where recycled water use is not permitted. However, proper preventative measures can reduce the risk to acceptable level. In the scenario where recycled wastewater effluent is too risky to be used on-site, off-site uses and/or discharge to surface water bodies are not representing an environmental hazard due to the quality of wastewater complying within the applicable guidelines.

Table A4.4. Comparison of the environmental impact and risk assessment at Sites D and G between the current conventional wastewater treatment and the proposed integrated microalgae cultivation process.

Conventional treatment	Integrated microalgae cultivation system
Wastewater treatment process	
• Primary treatments - screening and solid separation - dissolved air flotation for fat, oil and grease separation • Secondary treatments - anaerobic digestion (AD) in covered lagoon for COD and FOG removal - aerobic pond for nitrogen removal (Site G only) • Storage/evaporation ponds • Possible filtration of wastewater effluent before recycling	• Primary treatments - screening and solid separation - dissolved air flotation for fat, oil and grease separation • Secondary treatments - anaerobic digestion (AD) in covered lagoon for COD and FOG removal with biogas recovery - microalgae cultivation system on AD effluent for nitrogen and phosphorous recovery in algal biomass • Possible filtration of wastewater effluent before recycling
Hazards	
• Nitrogen • Phosphorous	• Nitrogen • Phosphorous • Algae biomass in wastewater effluent
Management and use of treated wastewater effluent	
• Storage in evaporation ponds • Irrigation of crops and pasture for fodder production	Uses of wastewater effluent • On-site uses (i.e., cleaning of yards, infrastructures and trucks, washing of animals other than final wash, animal drinking water, fire control, irrigation of gardens and green areas) • Off-site irrigation of crops and pasture for fodder production • Off-site discharge to surface water bodies Uses of algae biomass • On-site recycle to AD lagoon to improve biogas generation • On-site use as fertiliser on crops • On-site use as animal feed • Off-site use as pharmaceutical products • Off-site use as biofuel generation
Environmental endpoints	
• Soil • Surface water • Groundwater • Air	• Soil • Surface water • Groundwater • Wastewater treatment plant for on-site uses
Adverse environmental Impacts	
• Nutrient imbalance in soil causing toxicity and disease to plants and soils • Nitrogen contamination of groundwater • Eutrophication of surface water bodies due to nitrogen and phosphorous • Emission of greenhouse gases	• On-site contamination of potable water distribution system • Large land footprint

• Large land footprint	
Qualitative risk estimation	
• The concentration of nutrients in the treated wastewater effluents at Sites D and G (Table A4.2) exceed the AGWR guidelines (Table A4.1) • The likelihood of the identified environmental impacts relative to the uses of the treated wastewaters is considered ‘likely’ • The consequences of the identified environmental impacts can vary from ‘insignificant’ to ‘moderate’ • The risk associated with the management of the treated wastewater is classified as varying from *low* to *high* • Preventative measures are required	• The concentrations of nutrients in the wastewater effluent at Sites D and G (Table A4.2) comply with the AGWR guidelines (Table A4.1) • The wastewater effluent is not treated to potable standards • The consequences of on-site contamination of the potable water distribution system due to the recycle of non-potable wastewater effluent can vary from ‘minor’ to ‘major’ • The likelihood of on-site contamination of the potable water distribution system due to the recycle of non-potable wastewater effluent is considered ‘possible’ • The risk associated with on-site uses of wastewater effluent is *high* • Preventative measures are required
Preventative Measures	
• On-site treatment technologies aiming at reducing the nutrient content in the final effluent • Removal of nitrogen and phosphorous through specific technologies (chemical precipitation and nitrogen biological removal) • Recovery of nutrients in a usable form (e.g., fertiliser)	• Reticulation distributing recycled wastewater effluent needs to be separated from potable water distribution systems • Personnel training and proper Hazard Analysis Critical Control Point (HACCP) procedures need to be put in place as defined by the Australian Standards AS4696 2007 (Browne, 2007) to manage accidents and minimise risks • Avoid the reuse of the wastewater effluent in situation where contamination with potable water reticulation might happen
Qualitative residual risk estimation	
• The concentration of nutrients in the treated wastewater effluents at Sites D and G is expected to comply with the AGWR guidelines (Table A4.1) • The likelihood of the identified environmental impacts relative to the uses of treated wastewaters is expected to change from ‘likely’ to ‘unlikely’ • The consequences of the identified environmental impacts can vary from ‘insignificant’ to ‘minor’ • The residual risk is expected to be *low*	• The likelihood of on-site contamination of the potable water distribution system from the use of non-potable wastewater effluent is considered ‘rare’ • The consequences of on-site contamination of the potable water distribution system due to the recycle of non-potable wastewater effluent can vary from ‘minor’ to ‘moderate’ • The residual risk is determined as *low*

9.4.4 Review of LCA studies on microalgae cultivation

Life cycle assessment (LCA) studies are designed to consider all environmental impacts of a process, from ‘‘cradle to grave’’ (i.e., considers the sources of all inputs and fate of all products and wastes). LCAs are related to net energy analyses and are a very common tool used to assess the sustainability of a process. In his review of LCA studies on microalgae cultivation for biofuel production (Benemann et al., 2012), the worldwide renown algae expert John Benemann points out how most of the published studies are based on assumptions derived from experimental outcomes and theoretical knowledge of the process rather than from existing production systems, thus making the conclusions of the LCAs rather limited and problematic. The author suggests the need of LCA and techno-economic analyses to be developed together and in an iterative way, in order to envision and implement a feasible process that must operate within the constraints of existing practical and theoretical knowledge. This process will then set the objectives of R&D projects for pilot testing.

In their effort to reliably compare LCA studies and present the range of process possibilities and potential environmental impacts, Handler et al. (2012) presented a detailed examination of microalgae LCAs with a particular focus on the growth stage of algae in raceway ponds. Literature data and environmental impact factors were synthesized to focus on three main indicators, i.e., greenhouse gas (GHG) emissions, energy demand, and fresh water use for each literature source, all normalized to a consistent functional unit (1 kg of cultivated algae). In all cases, the environmental impacts of algae cultivation across all impact categories were highly variable, covering a range of over two orders of magnitude:

- GHG emissions per kg of cultivated algae range from 0.1 to 4.4 kg CO2eq., with the main contributors including electricity inputs for gas compression and movement, and pond water movement;
- Energy requirements per kg of cultivated algae vary by almost two orders of magnitude (0.154 – 14.59 MJ), most studies ranging between 0.6 and 2 MJ, with an average of 1.7 MJ;
- Makeup water to replenish for evaporation and leakage losses also varies widely between 1.3 and 105 L per kg of cultivated algae.

Recent sustainability studies have shown that the indirect energy input associated with nutrients supply constitutes a major energy cost and environmental burden during microalgae cultivation (Alcantara et al., 2013). However, the integration of microalgae cultivation systems with high nutrient wastewater effluents like abattoir AD effluents can significantly improve those environmental impacts associated with energy inputs for nutrient supply. Sander and Murthy (2010) performed an LCA study on a microalgae cultivation experiment for biodiesel production. Secondarily treated wastewater is used as a substrate for microalgae growth and it is assumed all nutrients needed for algae to grow are supplied from the wastewater (i.e., a similar scenario to the integrated microalgae process in abattoirs). The experiment is based on a 4 day harvest–growth–harvest cycle. The functional unit is 1,000 MJ of energy from algal biodiesel (24 kg of algal biodiesel). The authors quantified energy demand and CO_2 emission for the growth step in raceway ponds as equal to 15.43 MJ/functional unit and 0 kg/functional unit, respectively. The harvest and dewatering steps have a larger impact on energy demand and CO_2 emission (3,000 – 6,000 MJ for energy demand and 240 – 400 kg CO_2 emitted) depending on the used technology for algae concentration (filter press versus centrifuge). Through a LCA study on microalgae cultivation in raceway ponds, Clarens et al. (2010) demonstrated the advantages of using wastewater effluents as a substrate for algae growth. Average concentrations of total nitrogen and phosphorous equal to 25 and 7 mg/L, respectively, were measured in the growing medium. Although the nutrient content of the wastewaters used by Clarens et al. (2010) are not comparable with the wastewater effluents generated at Sites A, D and G, the study quantifies the improved environmental impacts from using wastewaters as a substrate for algae growth (Table A4.5). Substantial reductions in GHG emissions, energy and water use of the microalgae cultivation stage are found when wastewaters are used as a growth substrate (Table 6).

Table A4.5. Results of LCA on microalgae growth on a freshwater and wastewater substrate derived by Clarens et al. (2010). Results are expressed per functional unit (317 GJ biomass-derived energy) and refer to the cultivation stage in open raceway ponds.

	Freshwater	Wastewater (TN = 25 mg/L; TP = 7 mg/L)
GHG emission (kg CO_2eq.)		
Mean ± standard deviation	1.8 ± 0.59	1.1 ± 0.56
Percentage reduction relative to freshwater LCA (%)		39
Energy demand (MJ)		
Mean ± standard deviation	30 ± 6.6	2.4 ± 6.2
Percentage reduction relative to freshwater LCA (%)		92
Water use (m^3 x 10^4)		
Mean ± standard deviation	12 ± 2.4	9.4 ± 2.2
Percentage reduction relative to freshwater LCA (%)		22

Of particular interest for abattoir wastewater treatment plants equipped with a post AD nutrient removal stage (like Site G, for example), Clarens et al. (2010) found that the extremely energy-intensive nature of nutrient removal processes is likely to generate a higher energy burden than microalgae cultivation. About 60-80% of energy consumption during wastewater treatment is associated with nutrient removal, and wastewaters with higher nutrient concentrations are more environmentally burdensome to treat. Clarens et al. (2010) suggests that rerouting a portion of the nutrient load to algae cultivation is one way to reduce energy consumption during wastewater treatment, and also note that reductions in each life cycle impact category associated with avoidance of nutrient removal in wastewater treatment plants account for 50-70% of the total offsets. The conclusions of this study are quite relevant to abattoirs like Site G (where a nutrient removal process is currently adopted) as it shows how the environmental impacts (e.g., energy use, GHG emission and water use) of the wastewater treatment process could be minimized by integrating a microalgae cultivation step within the current system. A full LCA study on Site G abattoir is highly recommended once more data and an experimental study on algae bioprospecting and growth on the AD effluent are available. It is expected that the integrated microalgae process proposed in this project would significantly improve the environmental impacts of the operations at Site G abattoir.

The reviewed LCA studies are not directly inter-comparable because of different functional units defined in each study; however, they quantify the environmental impacts of microalgae cultivation and suggest the use of high nutrient wastewater effluents as a growth substrate to minimize the environmental impacts associated with GHG emission, energy use and water demand. In the context of abattoir operations and wastewater treatment, whether the microalgae cultivation system is grown on fresh or waste water, its environmental impacts in terms of GHG emission, energy demand and water use are expected to be significantly lower than the environmental impacts generated from abattoirs operations. The environmental performance review on Australian abattoirs published by

AMPC (Ridoutt et al., 2015) quantified the GHG emission, energy demand and water use of abattoirs per unit product (tonnes of HSCW) as an average of 432 kg CO2eq., 3,000 MJ and 8,000 L, respectively. The impacts on the environment of the cultivation of microalgae to treat the wastewater generated in abattoirs thus constitute a small fraction of the overall environmental impacts of the abattoirs.

9.4.5 Final use of the algae biomass and its environmental impact

Environmental impact assessments, techno-economic analyses and LCAs of microalgae cultivation systems are strongly related to the final use of the algae biomass. During the course of this project, a variety of off-site and on-site uses of the algae biomass product has been mentioned, e.g., biodiesel production, pharmaceutical products and oil extraction, animal feed, fertilizer and biogas production through recycle of algae biomass to AD. The most cost effective utilization of the algae biomass needs to be evaluated for each abattoir separately as it depends on the abattoir location, local market for algae products, process and techno-economic site assessment and conditions. It should be noted that the primary objective of integrating a microalgae cultivation step in the wastewater treatment process implemented at abattoirs is to reduce the environmental impacts associated with high nitrogen and phosphorus concentrations in the treated wastewater effluents currently generated in abattoirs. Thus the production of a nutrient-depleted water stream that can be recycled within the abattoir operations and/or safely discharge to the environment is the main end-product of the treatment process. In this context, the ability of microalgae cultivation systems to achieve high nutrient removal rates and generate a clean water stream is well understood in both the literature and practical applications. The production of clean water is then considered an achievable outcome and positive environmental impact of the process, due to the maturity of algae cultivation systems. In turn, negative environmental impacts are related to the energy demand, and subsequent GHG emissions, of the algae cultivation, harvesting and dewatering systems. In the context of wastewater treatment in abattoirs (where the most important end-product is a clean water stream), the grown algae biomass represents a process by-product whose inherent value can be exploited as a way to reduce and minimize the energy demand of the system. The perspective of our study substantially differs from the one found in the majority of LCA and environmental impact assessment studies where the main target is the conversion of algae biomass to biofuels. In the abattoir context, the final use of the biomass must therefore be related towards an environmentally sustainable and/or cost-effective solution. Uses of algae biomass for biofuel production are expected not to be competitive in the abattoir context due to specific process requirements that increase the energy demand of the cultivation and dewatering steps (e.g., high algae daily productivity and high biomass concentrations as percentage solids required for transportation and oil extraction). Technologies developed to produce bio-oil, e.g., such as hydrothermal liquefaction, that do not require dry algae biomass are not considered mature enough as fuel yields, quality and costs are yet to be determined (Benemann, 2013). Microalgae biomass revalorization through AD has been suggested as a promising process that involves AD on the raw algae biomass to produce methane as biofuel (Alcantara et al, 2013; Hernandez et al., 2016; Molinuevo-Salces et al., 2016; Polakovicova et al., 2012; Zhang et al., 2016). Because this process by-passes the algae concentration and oil extraction steps, both responsible for about 50% of production costs (Collet et al., 2011), it represents an attractive option for the biofuel industry. Digestion of pure algae biomass or co-digestion of algae and bacteria are both well studied in the literature as possible ways to maximize the methane yield (Wang and Park, 2015; Yuan et al., 2012; Alcántara et al., 2013). The production of electricity from biogas generated in the AD of algae biomass is expected to offset the energy demand of the cultivation, harvesting and dewatering steps, thus significantly improving the environmental impacts of entire system. Although attractive from the energy perspective, the re-valorization of algae biomass through AD in not compatible with abattoir wastewater systems as nitrogen and phosphorous are re-dissolved in the AD liquid digestate and a high nutrient stream is generated. Collet et al. (2011) and Ras et al. (2011) have determined the remineralization percentage of nutrients as varying with the hydraulic retention time (HRT) of the AD process, which highlights a trade-off between nutrient mineralization and methane production during AD of microalgae *Chlorella vulgaris*. An HRT of 16 days caused 19% of the nitrogen content in biomass to mineralize and a methane conversion rate of 147 mL CH_4/g VSS (Ras et al., 2011). An HRT of 28 days caused 68% of the nitrogen content in the algae biomass to dissolve, although the methane conversion rate increased to 240 mL CH_4/g VSS (Ras et al., 2011). A 90% nitrogen mineralization and a methane conversion of 292 mL CH_4/g VSS was suggested by Collet et al. (2011) at an HRT of 46 days.

Collet et al. (2011) undertook a LCA and environmental assessment of the use of methane from algae as a biofuel. The system is composed of raceway ponds, a dewatering system by settling and centrifugation with the concentrated algae biomass sent to an AD reactor for biogas production. The water from the algae concentration step and the liquid digestate from AD are recycled back to the ponds to make up for evaporation and water losses. A fraction of the biogas (about 26%) is recycled within the AD process as heat, with the rest of the biogas being purified to methane and used to produce electricity. CO_2 is provided to the algae ponds from external sources and nutrients from added fertilizers are recovered from the recycle liquid digestate. Collet et al. (2011) estimated the total electric consumption equal at 0.64 kWh/kg of algae (3.2 kWh/m^3 of methane and 16,000 kWh/d) for the whole system and the most consuming stages as:

- the paddlewheels: 31.2%
- the pumping between the ponds and the settlers: 23.9%
- the anaerobic digestion plant: 20.8%, with 16.9% for the mixing of the digesters and the pumping, and 3.9% for the centrifugation of the digestates
- the purification plant: 13%
- the centrifugation of the algae: 6.6%
- the CO2 injection: 4.5%

Although attractive for its ability to generate electricity and offset the energy demand of the algae cultivation, harvesting and dewatering process, the AD of the whole biomass produced daily is impractical in the selected abattoirs due to the remineralization of nitrogen and phosphorous. The use of biomass as fertilizer and animal feed is possibly the most appropriate option at the selected abattoirs. These uses will translate in potential revenue and/or economic savings for the abattoirs operations, and possibly offset the cost associated with the energy demand of the cultivation and dewatering system. The use of algae biomass as protein supplement in animal feed has been widely suggested and researched as a sustainable way to help address the global energy, food and environmental crisis and the current competition for resources (e.g., land and water) between animal feed and human food supply (Benemann, 2013; Lum et al., 2013; Bleakley and Hayes, 2017). Recent estimates indicate that 30% of the global algal production is used by the animal feed industry and, although their nutritional profiles vary considerably with the species used, a large majority is characterized by protein, carbohydrate, and lipid contents that are comparable, if not superior, to conventional feeds (Lum et al., 2013). Lum et al. (2013) reviewed several applications of using sewage-grown *Chlorella* and *Scenedesmus* (both algae that have been demonstrated to grow well on high nutrient wastewaters; Nwoba et al., 2016, and Ayre et al., 2017) to supplement the diet of chickens due to their high crude protein and carotenoid contents. In order to make it more digestible for use as animal feed, the harvested biomass has to go through cell breakage to extract the protein content (Benemann, 2013). A process that has been recently suggested in the literature is the integration of biogas production and animal feed through microalgae: the protein content of the algae biomass can be extracted and used as animal feed, whilst the remaining fraction that is high in carbon and lipids can be anaerobically digested to produce methane (Lum et al., 2013; Benemann, 2013). By this process, the use of microalgae biomass in animal feed is expected to not only improve human and animal food security, but also facilitate cost-effective biogas production and reduces greenhouse gas production (Lum et al., 2013, and references therein). Such process could be an attractive option for abattoirs as it would avoid the remineralization of nitrogen and phosphorus during the AD of algae biomass while producing electricity from biogas.