Bio-upgrading piggery biogas by growing algae, for value-add end uses

Project 4C-119

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Australasian Pork Research Institute Limited
(APRIL)

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1 Executive Summary

Anaerobic ponds are a common effluent treatment technology used to simultaneously treat and store effluent whilst also producing biogas that can be harnessed if the ponds are covered with an impermeable plastic cover. This biogas is rich in methane (CH_4), with the remainder constituents being carbon dioxide (CO_2) and other trace gases. Uncovered anaerobic ponds are installed at an estimated 60% of Australian piggeries, and in recent years there has been strong uptake of biogas capture and use systems at various piggeries around Australia. When captured and burned, the CH_4 content of biogas represents a source of renewable heat and electricity. The main drivers for the increased uptake of biogas collection and use systems have been rapidly increasing on-farm energy costs, an industry commitment to reduce greenhouse gas (GHG) emissions (Co-operative Research Centre for High Integrity Australian Pork target: 1 kg CO_2-eq per kg hot standard carcass weight [HSCW] of pork produced), and the introduction of the Federal Government's Carbon Farming Initiative (now Emissions Reduction Fund or ERF) with financial incentives (i.e. through carbon credits) for producers to capture and utilise manure management CH_4.

Raw biogas from piggeries contains 60-70% CH_4, 30-40% CO_2, 0.07 – 0.40% hydrogen sulphide (H_2S), moisture and other trace components. Most of the existing biogas use systems at Australian piggeries utilise combined heat and power (CHP) systems and in many cases, the volume of biogas produced at a piggery exceeds the volume required to meet on site electrical requirements. This results in excess biogas being flared (essentially no value recovered), or exporting of excess electricity to the grid at relatively low rates of return.

Upgrading biogas to reduce H_2S, reducing CO_2 and achieving >90% CH_4 methane can both decrease the cost of biogas usage and can expand the usage options for biogas to include transport fuels (after compression) or the export of biogas into centralised natural gas grids, thereby improving overall on-farm energy use and/or maximise economic returns from biogas. Globally, technologies for upgrading biogas are immature, complex and not commercially viable at scales relevant to many piggeries. This project aimed to develop and test novel microbial technologies with the potential to upgrade biogas at smaller scale and lower cost. Specifically, the project aimed to develop purple phototrophic bacteria as a technology to remove the H_2S content of biogas and microalgae as a technology to remove the CO_2 content of biogas, thereby increasing the concentration of methane.

The project successfully demonstrated a continuous biogas desulphurisation process using purple phototrophic bacteria (PPB). The PPB process can run chemical-free and can integrate with existing anaerobic pond technologies. The PPB process achieved an average H_2S removal of 69-77% in the continuous process, with a maximum removal of 90%. The removal efficiencies achieved by the PPB system reduced H_2S to levels suitable for on-farm uses such as boilers and CHP units. The PPB technology therefore represent an alternative to conventional commercial iron oxide pellet scrubbers for on farm use, and does not introduce nitrogen as an impurity as does micro-aeration-based biological scrubbing systems. However,

multi-stage reactors or a secondary treatment step would be required to achieve complete H_2S removal for downstream use as a transport fuel and/or export into natural gas grids.

However, the cost of the PPB process was estimated at approximately \$85 kgS^{-1}; which is prohibitively high compared to competing H_2S removal technologies. The major cost for the PPB process is the electricity used to irradiate/illuminate the reactor overnight. Further work is needed to understand how the process would operate under light-dark-cycling conditions using only sunlight, and thereby eliminating the expensive illumination costs. Also, the availability of excess biogas-derived electricity may improve the overall economics of the process. Protein-rich biomass was generated, but in very small amounts which would also not be sufficient to offset the high costs of treatment.

The project successfully demonstrated at batch a CO_2 removal technology using the marine microalgae *T. suecica*. This microalgae technology was successful at removing both CO_2 from synthetic biogas as well as utilising some nutrients from a piggery pond effluent medium. The process achieved a CO_2 removal up to 94%. When applied to piggery biogas; the treated biogas could have a CH_4 content of 94 to 98%, making the upgraded gas suitable for use as a transport fuel (after compression) or to export biogas into centralised natural gas grids. Due to the high nutrient content of piggery effluent, only a small portion of the available effluent nutrients (~6% nitrogen and ~1% phosphorous) were required to support microalgal growth and sequestration of CO_2. Production costs of the microalgal biomass have not been determined at this stage. The next stage of development could be to explore a continuous process, possibly at pilot stage, to clarify the biomass yields, carbon uptakes rates and harvesting costs to allow a more detailed assessment of the viability of biogas-based microalgae cultivation systems.

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2 Introduction

2.1 Background

Anaerobic ponds are a common effluent treatment technology used to simultaneously treat and store effluent whilst also producing biogas that can be harnessed if the ponds are covered with an impermeable plastic cover. This biogas is rich in CH_4, CO_2, and other trace gases. Uncovered anaerobic ponds are installed at an estimated 60% of Australian piggeries and in recent years there has been strong uptake of biogas capture and use systems at these facilities. In 2018, approximately 15% of the total Australian pig herd (42,700 sows ≈ 427,000 SPU) was housed in piggeries where the effluent is directed to a biogas system. This is equivalent to 29% of the national herd housed in accommodation currently considered 'suitable' for biogas system adoption (excluding deep litter housing, outdoor production and piggery units with capacities less than 500 sows farrow to finish). This corresponds to biogas systems operating at 21 piggery units across Australia, representing 15 separate businesses (Tait, Pork CRC project 4C-116; Final Report).

When captured and burned, the CH_4 content of biogas represents a source of renewable heat and electricity. The main drivers for the increased uptake of biogas collection and use systems have been rapidly increasing on-farm energy costs, an industry commitment to reduce greenhouse gas (GHG) emissions (Co-operative Research Centre for High Integrity Australian Pork target: 1 kg CO_2-eq per kg hot standard carcass weight [HSCW] of pork produced[1]), and the introduction of the Federal Government's Carbon Farming Initiative (now Emissions Reduction Fund or ERF) with financial incentives (i.e. through carbon credits) for producers to capture and utilise manure management CH_4.

The majority of the existing biogas use systems at Australian piggeries utilise combined heat and power (CHP) systems which i) generate electricity for on-farm use, with the potential to export surplus electricity to the grid); and ii) capture waste heat in the form of hot water, which is then used to heat farrowing and weaner sheds. Both total biogas energy availability and the mixture of energy uses at Australian piggeries can vary based on size, location, design, time of year and other factors. Consequently, in many cases, the volume of biogas produced at a piggery exceeds the volume required to meet onsite energy requirements, and this results in excess biogas being flared (essentially no value recovered) or exporting of electricity to the grid at relatively low rates of return.

Raw biogas from piggeries contains 60-70% CH_4, 30-40% CO_2, 0.07 – 0.40% H_2S, moisture and other trace components. Upgrading biogas to reduce H_2S, reduce CO_2 and achieve >90% CH_4 has a higher treatment cost, but can both decrease the cost of biogas usage and can expand the usage options for biogas to potentially include transport fuels (after compression) or the export of the purified CH_4 into centralised natural gas grids. The higher CH_4 content in treated biogas can also up-rate generator engines (more electricity from the same engine size, or allow use of a smaller engine which can cost less). For this reason, the cleaned biogas can also be easily used at piggeries that already harness biogas, to get more electricity out of

[1] https://porkcrc.com.au/research/

existing infrastructure. Therefore, upgrading biogas potentially improves overall on-farm energy use and/or maximise economic returns from biogas.

2.2 Biogas Generation at Piggeries

2.2.1 Waste Effluent Generation

Piggery effluent includes faeces, urine, spilt feed, split water from drinkers, water from wash down and flushing systems. The waste effluent is highly odorous and turbid. Figure 1 [6] summarises common by-product management options for conventional piggeries. Common effluent management processes include solids separation, before effluent is treated and recycled. The primary goal of the effluent treatment system is to reduce organic content in liquid effluent, although nitrogen reduction through ammonia volatilisation is an added consideration. Treatment ponds are an integral part of effluent treatment at most piggeries due to low-cost construction and simple, robust operation. The ponds maybe anaerobic, facultative, aerobic and in some cases, emergency wet-weather and evaporation systems. Many primary effluent treatment ponds are anaerobic and produce i) fugitive CH_4 which, if the pond is covered with an impermeable plastic cover, can be harnessed as a biogas stream to be utilised for energy production; and ii) a stabilised sludge that accumulates at the bottom of the pond and must be removed periodically. The presence of screens is known to remove solids prior to the ponds and reduce biogas yields. The type of lagoon present may also reduce waste nutrients available to support biomass growth in downstream processes (i.e., increased volatilization of nitrogen in facultative or holding ponds).

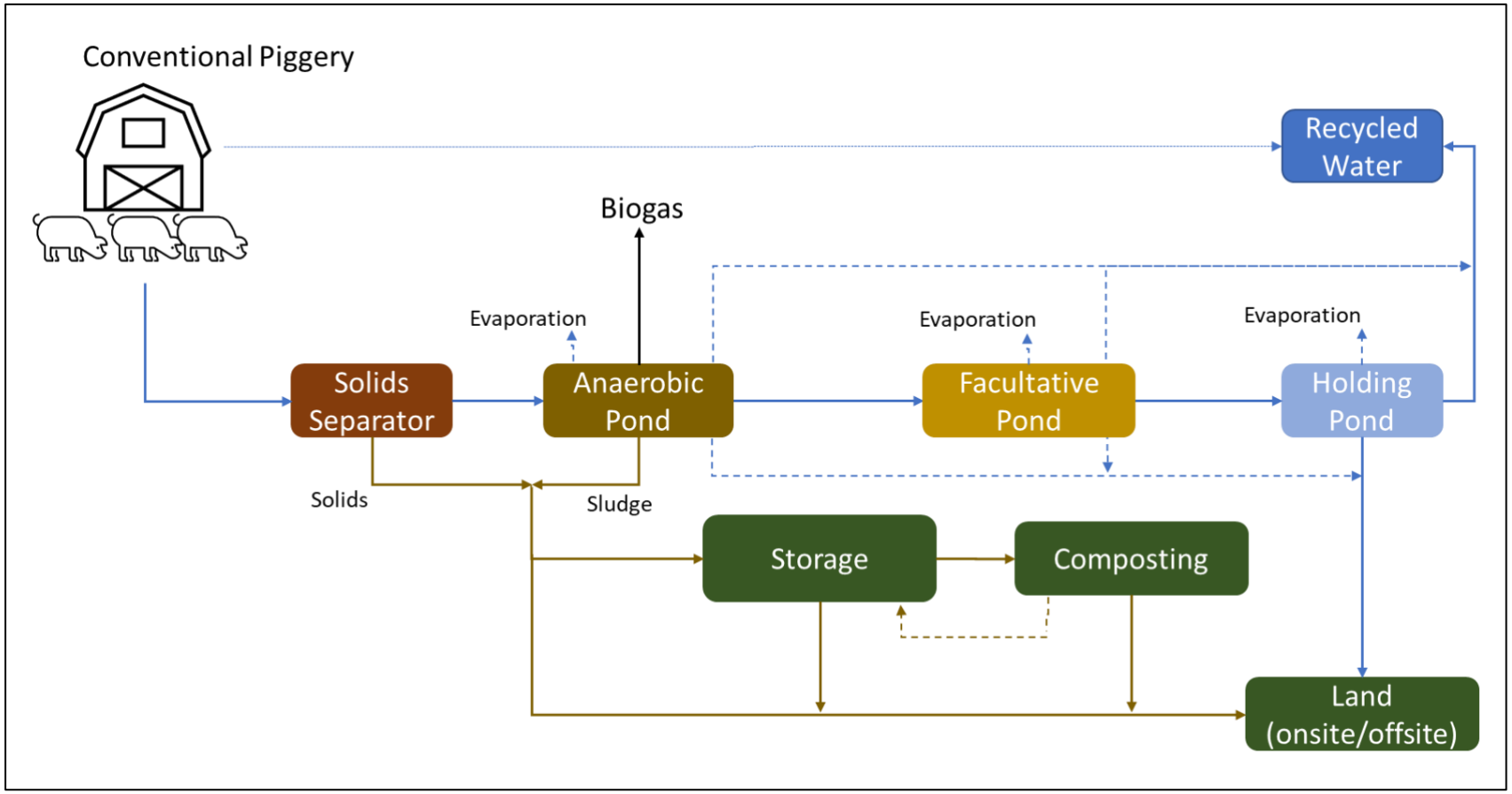

Figure 1: Effluent and by-product management at piggeries (adapted from [6]).

There are a number of factors that influence the quantity and quality of waste effluent produced including diet, feed intake, feeding technique, climatic conditions, effluent handling systems employed [1]. Table 1 shows the outputs for various pig classes and standard pig unit (SPU) multipliers.

Piggery effluent is typically characterised according to solids content (total and volatile), nitrogen (total and ammoniacal), phosphorous, volatile fatty acids and Chemical Oxygen Demand (COD, total and soluble). Of these metrics, the mass of volatile solids is typically an indicator of biogas production. Table 2 shows the characteristics of piggery effluent reported by Gopalan [2], which included a range of piggery units covering different production stages. The data from [2] was used to estimate the ratios of nitrogen, phosphorous and solids in piggery effluent; these ratios have then been applied to estimate the required nutrient removal during biological biogas treatment.

Table 1 Standard pig unit (SPU) multipliers and VS outputs for a range of pig classes [3] adapted from [4].

Pig Class	Live Weight Range (kg)	Age Range (weeks)	SPU ($SPU.pig^{-1}$)	VS Output ($kgVS.pig^{-1}.yr^{-1}$)
Gilts	100 – 160	24 – 30	1.8	162
Boars	100 – 300	24 – 128	1.6	151
Gestating Sows	160 – 230	-	1.6	151
Lactating Sows	160 – 230	-	2.5	215
Suckers	1.4	0 – 4	0.1	11.0
Weaner pigs	8.0	4 – 10	0.5	47
Grower pigs	25.0	10 – 16	1.0	90
Finisher pigs	55.0	16 – 24	1.6	149
Heavy finisher pigs	100.0	24 - 30	1.8	-

Table 2 Characteristics of piggery effluent streams from sheds holding different production stages (adapted from [5])

Waste Stream	Source	TS*	VS*	VS	TKN	TP	TKN/VS ratio	TP/VS ratio
		(g L^{-1})	(g L^{-1})	(%TS)	(g L^{-1})	(g L^{-1})		
Dry sows	FFA1	49±3	37±2	37	3.4	0.6	0.09	0.02
	FFA2	20±2	13±2	13	2.1	0.9	0.16	0.07
	FFB	32±4	22.±4	22	1.9	1.1	0.09	0.05
	FFC	23±4	18±3	18	1.7	0.07	0.09	0.00
	BR	69±2	43±1	43	3.1	1.7	0.07	0.04
Farrowing	FFA1	35±10	23±6	23	2.6	0.6	0.11	0.03
	FFA2	21±1	15±1	15	2.5	0.6	0.17	0.04
	FFB	17±2	12±2	12	1.7	0.5	0.14	0.04
	FFC	35±6	28±6	28	2.6	0.3	0.09	0.01
	BR	19±1	12±1	12	1.5	0.3	0.13	0.03
Weaner	FFA1	42±10	35±8	35	3.2	1	0.09	0.03
	FFA2	17±1	14±1	14	2.1	0.5	0.15	0.04
	FFC	27±10	24±9	24	1.7	0.2	0.07	0.01
	GO	19±3	15±3	15	0.8	0.3	0.05	0.02
Grower	FFA1	41±5	34±5	34	2.6	0.6	0.08	0.02
	FFA2	20±1	15±1	15	2.9	0.5	0.19	0.03
	FFB	51±7	43±7	43	3.7	0.5	0.09	0.01
	GO	37±8	29±8	29	2.5	0.6	0.09	0.02
Finisher	FFA1	37±3	29±2	29	2.6	0.6	0.09	0.02
	FFA2	17±1	12±1	12	4.2	0.7	0.35	0.06
	FFB	37±8	30±6	30	2.5	1.2	0.08	0.04
	FFC	60±20	50±18	50	2.5	0.5	0.05	0.01
	GO	30±3	23±2	23	2	0.4	0.09	0.02
Composite	FFB	15±5	11±4	11	2.5	0.6	0.23	0.05
	FFC	20±3	15±2	15	2	0.3	0.13	0.02
	GO	29±5	23±4	23	1.5	0.4	0.07	0.02
						Min	0.05	0.00
						Average	0.12	0.03
						Max	0.35	0.07
*Results presented as value±95%error for TS, VS (replicates of three) and TCOD (replicates of five).								

2.2.2 Biogas Volume and Composition

Skerman [3] presented estimates for VS, biogas and methane generation at Australian piggeries using covered anaerobic ponds, the results of which are summarised in Table 3. Biogas production of 30 $m^3.SPU^{-1}.yr^{-1}$ was a conservative estimate for a piggery that was screening effluent prior to the pond, and biogas production of up to 50 $m^3.SPU^{-1}.yr^{-1}$ could be possible for a piggery without effluent screening. The conservative estimate for biogas production corresponds to a

conservative estimate for CH_4 production of 19 m^3 CH_4 per SPU per year, with the potential to generate 177 kWh (637 MJ) of primary energy per SPU annually.

Using the figures above the average daily gas production for an Australian piggery can be estimated at 0.08 $m^3.SPU^{-1}.d^{-1}$; however, daily biogas production volumes will vary between summer and winter seasons. Birchall [7] reported seasonal biogas variations in the range of 20%; when sizing biogas treatment and utilization systems this variation would be considered in order to ensure the system can accommodate the higher summer biogas production. Therefore, the maximum daily biogas production during summer would be 0.16 $m^3.SPU^{-1}.d^{-1}$ (for 50 $m^3.SPU^{-1}.yr^{-1}$) or 0.10 $m^3.SPU^{-1}.d^{-1}$ (for 30 $m^3.SPU^{-1}.yr^{-1}$); for a 500 SPU piggery this corresponds to summer biogas production of 82 $m^3.d^{-1}$ (for 50 $m^3.SPU^{-1}.yr^{-1}$).

Typical properties of raw biogas from Australian piggeries are shown in Table 4. Raw biogas from piggeries contains 60-70% CH_4, 30-40% CO_2 and 0.07 – 0.40% H_2S.

Table 3 Estimated volatile solids, biogas and CH_4 production from covered anaerobic ponds at Australian piggeries [3].

Parameter	Units	Conservative	Maximum
VS Production	$kgVS.SPU^{-1}.yr^{-1}$	90	110
Static Screen VS removal	%	25	25
Residual VS after Screening	$kgVS.SPU^{-1}.yr^{-1}$	68	83
Biogas Yield	$m^3.kgVS^{-1}$	0.43	0.46
Biogas Production (no Screen)	$m^3.SPU^{-1}.yr^{-1}$	38.7	50.6
Biogas Production (Screen)	$m^3.SPU^{-1}.yr^{-1}$	29.2	38.2
Biogas Composition (%CH_4)	% CH_4	65	65
Methane Yield	$m^3.kgVS^{-1}$	0.28	0.30
Methane Production (no Screen)	$m^3.SPU^{-1}.yr^{-1}$	25.2	32.9
Methane Production (Screen)	$m^3.SPU^{-1}.yr^{-1}$	19.0	24.8

Table 4 Typical properties of raw biogas from Australian piggeries [8]

Parameter	**Range**
Heating Value (MJ/m^3)[a]	18 -24
Density (kg/m^3)[b]	1-1.2
Explosive Atmosphere range (% biogas in air)[b,c]	5-24
Raw biogas temperatures	10-70
Methane (CH_4) % by volume	60-70
Carbon dioxide (CO_2) % by volume	30-40
Hydrogen Sulphide (H_2S) % by volume	0.07-0.40
Hydrogen Sulphide (H_2S) as ppm by volume	700 – 4000

(a) Influenced by moisture and methane content.
(b) Influenced by methane and carbon dioxide content.
(c) The lower explosive limit for pure methane in air is 5% by volume, which was taken as a conservative estimate for piggery biogas. The upper explosive limit of 24% by volume in air reflects the dilution effect of carbon dioxide.
Source: Ross, C.C. and Walsh, J.L. (1996). Handbook of Biogas Utilization. US Department of Energy.

2.2.3 Treatment and Utilization of Biogas

Figure 2 is an example of the typical biogas treatment use steps at an Australian piggery. This system includes treatment steps to reduce the concentration of H_2S and cooling of the biogas and moisture traps to reduce the water content. In most of the existing biogas use systems, the biogas is then burned using combined heat and power (CHP) systems which i) generate electricity for on-farm use, with the potential to export surplus electricity to the grid); and ii) capture waste heat in the form of hot water, which is then used to heat farrowing and weaner sheds.

Raw biogas from piggeries contains H_2S at concentrations ranging from 700-4000 ppm (volume basis), corresponding to sulphur loads of 0.048 to 0.27 $kgS.SPU^{-1}.yr^{-1}$. H_2S is a toxic trace gas, with potential human health effects from exposure to H_2S summarised in Table 5. H_2S is also a corrosive gas which at typical concentrations in piggery biogas will likely require removal from the gas before usage; the removal of H_2S from biogas occurs through desulphurization.

Raw biogas will also be saturated with water vapour that must be removed prior to use. The moisture content of biogas can be estimated from humidity charts at approximately 17 g water per m^3 of biogas at 20°C, increasing to 40 g water per m^3 of biogas at 35°C and biogas temperatures under an impermeable pond cover can be as high as 60°C or more due to solar radiation heat on the cover.

Table 5 Potential human health effects from short-term exposure to H_2S [8]

H_2S content of a gas mixture	**Human health effects after short term exposure**
0.003 – 0.02 ppm	detectable "rotten egg" odour
10 ppm	causes eye irritation and chemical changes to blood and muscle tissue
100 ppm	causes loss of sense of smell so an exposed person does not know that conditions are dangerous
320 – 530 ppm	causes fluid accumulation in the lungs with risk of death
500 – 1000 ppm	causes rapid breathing then loss of breathing
1000 ppm	causes nervous system failure
700 – 4000 ppm	typical concentration in piggery biogas - causes nervous system failure
*Warning alarms on many handheld gas safety detectors are set at 10ppm signifying safe short term exposure levels. Sources: Department of Health New York USA. Health Effects from Inhalation of Hydrogen Sulphide; Skrtic, L. Hydrogen Sulphide, Oil and Gas, and People's Health. 2006.	

Table 6 Recommended maximum H_2S concentrations in biogas used in a range of applications [9, 10].

Application	**Recommended maximum biogas H_2S concentrations (ppm)**
Heating (Boilers)	1,000
Internal Combustion Engines (CHP)	200 – 1,000
Micro-turbines	10,000 – 70,000
Centralised natural gas grids	4

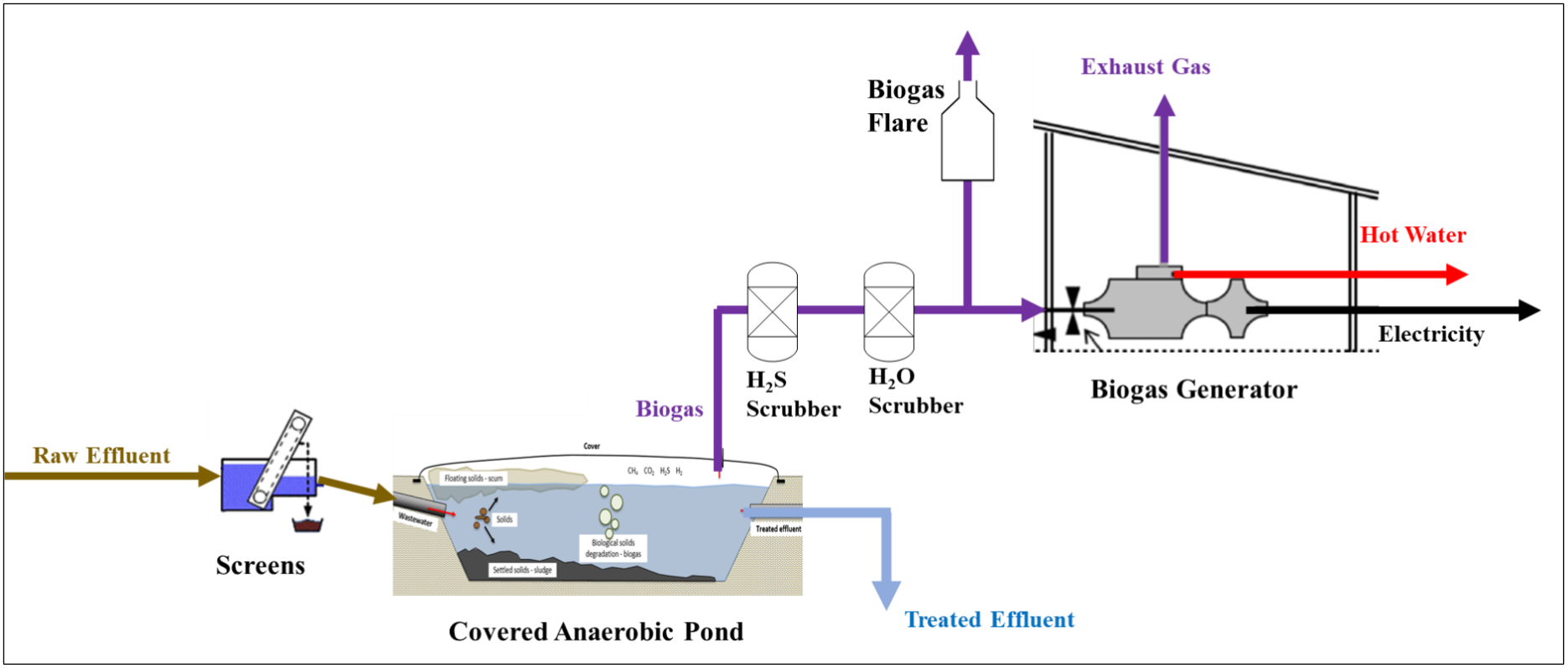

Figure 2 Typical treatment steps in a piggery biogas plant with a covered anaerobic lagoon (Adapted from [8])

2.3 Biogas Treatment - Overview

Biogas treatment and upgrading is a rapidly developing technology area. There are a broad range of technologies either commercially available or progressing through laboratory and pilot scale development. An example list of biogas upgrading technologies is shown in Table 7, with a more detailed review presented by Munoz [9]. To date, many commercially available methods rely on complex sequences of physical/chemical technologies, which due to high energy consumption and chemical usage are not environmental and economically feasible at scales relevant to Australian piggeries. The high cost and complexity of existing technologies has triggered the rapid development of biological technologies targeting improved economic and environmental performance.

Table 7 Technologies for physical/chemical or biological upgrading of biogas [9, 11]

	Desulphurization (H_2S removal)	Decarbonization (CO_2 Removal)
Physical/Chemical Technologies	• *In situ* precipitation • Adsorption • Water Scrubbing • Organic Solvent Scrubbing • Chemical Scrubbing • Membrane Separation	• Water Scrubbing • Organic Solvent Scrubbing • Chemical Scrubbing • Pressure Swing Absorption • Membrane Separation
Biological Technologies	• Biofiltration of H_2S • *In situ* microaerobic H_2S oxidation • Chemoautotrophic sulphide oxidation • Oxygenic Photosynthetic H_2S removal (algae) • Oxygenic Photosynthetic H_2S removal (PPB)	• Chemoautotrophic biogas upgrading • Photosynthetic biogas upgrading • Enzyme catalysis • *In situ* desorption

2.4 Desulphurization and Purple Phototrophic Bacteria (PPB)

There are a range of existing commercial technologies for desulphurisation available. A common requirement in these technologies is the transfer of H_2S from the gaseous phase (biogas) into either a liquid phase (via absorption), or onto a solid carrier (via adsorption). In the case of biological processes, the H_2S is then oxidised through a series of biological reactions, and thereby removed [12].

Chemical removal of sulphide can be achieved with regenerable absorbents in an amine solution with a multistep regeneration process [13, 14]. Other removal methods are non-regenerable absorbents (e.g. Fe/Zn oxidation) or direct conversion of sulphide to elemental sulphur via combustion [12]. However, all chemical

removal technologies require large amounts of chemicals and energy, with chemical input costs scaling with size [15].

Biological sulphide oxidation with sulphide oxidising bacteria (SOB) in a micro-aerated tank after absorption in a separate column, is a relatively new commercial biotechnology applied for H_2S removal technology e.g. THIOPAQ® [16]. However, the capital costs of such a two-column system can be significant because the regeneration reactions for elemental sulphur are highly exothermic and the value of elemental sulphur as a commodity chemical is low. Micro-aeration applied directly to the anaerobic digester oxidises sulphide to elemental sulphur with low operating costs, but introduces nitrogen as a contaminant into the treated gas [17].

Phototrophic microorganisms such as algae [18] and green sulphur bacteria (GSB) have been used to remove sulphide from sour gases, wherein oxygenic algae introduce oxygen into the treated gas for sulphide oxidation. Anoxygenic GSB have been investigated extensively for their ability to remove sulphide from liquid or gaseous streams, mainly for odour control purposes [19]. However, removal rates differed noticeably between the different systems and a biomass-specific removal rate has not been previously compared. This makes an assessment of process feasibility at larger scale difficult. Both organisms require irradiation in the visible light spectrum, which, depending on reactor footprint, can contribute to high energy costs for treatment [20].

Recently, anoxygenic purple phototrophic bacteria (PPB) have gained attention for complete nutrient recovery from wastewater and have been found to be versatile in terms of metabolism and robust in process applications [21, 22]. Many species of PPB can grow photo-autotrophically using sulphide as electron donor to utilise inorganic carbon (e.g., CO_2) as the carbon source for biomass growth [23-26]. This makes PPB a promising candidate for H_2S removal with simultaneous CO_2 removal. However, the theoretical ratio of S to C removal is 0.5 (mole basis) [19], and because the proportion of CO_2 in bigas (30-40%) is substantially greater than H_2S (0.4%), the removal of H_2S from biogas would only minimally reduce the CO_2 concentration by about 0.8%.

In contrast to algae and GSB, PPB absorb light in the infra-red (IR) spectrum, potentially lowering the energy requirements of irradiation. For example, applying IR-light, Marin et al. [27] successfully used PPB to simultaneously treat piggery effluent and upgrade biogas, including complete sulphide removal. However, this process is partially heterotrophic as well as autotrophic. Fully autotrophic PPB (using H_2S only as electron donor) has a number of potential advantages, including: lower light energy inputs compared to phototrophic processes using the visible spectrum, with potential to instead use the IR spectrum of sunlight; recovery of a pure PPB product; and maximal removal of CO_2 from the gas stream. In addition, anoxygenic photosynthesis does not generate oxygen. In practice, this would involve a reactor fed with high sulphide feed streams for sulphide removal, but it is necessary to identify the basic capability and removal mechanism of mixed culture PPB on autotrophic (sulphide) feeds.

2.5 Carbon Dioxide Removal and Microalgae

Piggery biogas typically contains 60-65% CH_4 (by volume). To use biogas as a transport fuel (after compression) or to export biogas into centralised natural gas grids, the CH_4 content needs to be increased to greater than 90% and in some applications greater than 95% [28].

There are a range of mature commercial technologies for removal of CO_2 from natural gas, landfill gas and biogas. Currently, commercial technologies are based on physical and/or chemical processes such as scrubbing with water, organic solvents or chemicals, pressure swing absorption, cryogenic CO_2 separation or membrane separation [9]. Commercial physical/chemical processes for CO_2 removal can be designed at various scales, but are generally applied for biogas flowrates ranging from 200 $m^3.h^{-1}$ to above 1000 $m^3.h^{-1}$ due to the complexity of the process and for economic reasons. These biogas flow rates would correspond to a 40,000 SPU facility (200 $m^3.h^{-1}$) to greater than a 200,000 SPU facility (1,000 $m^3.h^{-1}$).

Microalgae are prokaryotic or eukaryotic, unicellular or simple multicellular photosynthetic microorganisms, that exhibit high photosynthetic efficiency, rapid growth rate (doubling as short as 3.5 hours), and are capable of year-round production. Microalgae are autotrophic microorganisms that utilise CO_2 as the carbon source for growth. Growth of microalgal photosynthesis is well documented as one of the most economical ways to sequester CO_2 [29], fixing approximately 1.83 kg of CO_2 to generate 1 kg of dry algal biomass [30]. Microalgal photosynthesis is well documented to be one of the most economical ways to sequester CO_2 [29]. Microalgae could effectively generate 1 kg of dry algal biomass by bio-fixing approximately 1.83 kg of CO_2 [30].

Microalgae have been estimated to have higher biomass productivity than plant crops in terms of land area required for cultivation [30] and are well-recognized as an alternative to existing biofuel producing crops such as corn and soybean [31]. Apart from lipids, microalgae can also produce a large range of valuable co-products, such as fats, polyunsaturated fatty acids, oil, natural dyes, carbohydrates, pigments, antioxidants, high-value bioactive compounds, and other fine chemicals and the biomass can even be used as feed/fertilizer [32]. In spite of its inherent potential, cost-effective and sustainable production of microalgal biomass at commercial scale has to date been impeded by various challenges, such as the requirements for large quantities of water and nutrients.

As microalgae require significant amounts of CO_2 for growth, there is potential to use biogas as the CO_2 supply [33]. Additionally, microalgae are able to use piggery effluent (after anaerobic pond treatment) as a source of nitrogen and phosphorous for growth. Integrating microalgae systems for on-farm treatment of biogas and waste effluent strategies could help make both microalgal biomass production systems and the host farm systems more sustainable, eco-friendly and cost-effective. To date, studies on effluent-based microalgal biomass production utilizing biogas as source of CO_2 focused mainly on freshwater species [34].

There are risks that salinity levels can become elevated in these systems, for example if (1) using moderate salinity groundwater as a water source onsite; and/or

(2) if treated liquid effluent is being repeatedly recycled for in-shed flushing of animal housing to reduce overall water use onsite, thereby causing a progressive build-up of salt from evaporation in uncovered effluent treatment ponds [35, 36].

In Australia, freshwater is a limited commodity and therefore cultivating microalgae using freshwater could be unrealistic [37]. Few studies have applied salt tolerant or marine microalgae [38-40]. However, *Tetraselmis sp*. is a marine algae that has been found to endure high concentrations of CO_2 and can be cultivated in wastewaters [41, 42]. Marine microalgae is an attractive alternative, potentially providing similar benefits to freshwater microalgae, e.g. high biomass productivity, high oil content, and ability to tolerate high nutrient content and CO_2 concentrations [43]. Additionally, a saltwater-based microalgae production system was found to have up to a 90% reduction in freshwater requirements [44] and could tolerate the salinity typical of many agricultural production effluents, if effluent was used as a cost-effective nutrient and water source for the microalgal growth.

2.6 Project Aims and Structure

This project aimed to test and develop technologies which bridges three active research areas, namely (1) biogas, (2) PPB and (3) microalgae. The project was a collaboration between Murdoch University (algae) and The University of Queensland (biogas, PPB). The core goal of the technologies was to remove H_2S and CO_2 from piggery biogas to improve the safety and useability of piggery biogas and to increase CH_4 concentration (not amount), thereby increasing "biogas quality". The high-quality treated biogas has expanded uses such as (after compression) vehicle fuel in tractors or in trucks for transporting pigs.

Aims related to the development of PPB technology to remove H_2S from biogas were to:

1. Investigate a fully autotrophic process for sulphide removal via PPB to estimate sulphide removal rates as well as biomass yield.
2. Demonstrate PPB based sulphide removal in a continuous process and identify important design parameters for a full-scale process.
3. Assess the viability of a continuous PPB based sulphide removal process, including comparison to existing desulfurization technologies.

Aims related to development of algae technology to remove CO_2 from biogas were to:

1. Integrate effluent treatment with biogas purification using a saline microalga, which has not been done before.
2. Determine the growth characteristics, biomass composition and maximum quantum yields marine microalga *Tetraselmis suecica* using synthetic biogas as a source of CO_2, and ADPE as a source of nutrients.
3. Explore the impact of pH and the resulting CO_2 partial pressure on microalgae growth
4. Assess the effect of CH_4 in biogas on microalgal cultivation of *Tetraselmis sp*.

3 Biogas Desulfurization using Purple Phototrophic Bacteria

The work in this section was completed by Felix Egger at The University of Queensland, resulting in the PhD thesis:

Egger, F. 2021. Gas phase production of mixed culture phototrophic bacteria. Thesis submitted for the degree of Doctor of Philosophy, School of Chemical Engineering, The University of Queensland.

The supporting research team included: Tim Huelsen, Stephan Tait, Paul D Jensen and Damien J Batstone.

The batch experimental component was also published in the following peer-reviewed journal paper:

Egger F, Hülsen T, Tait S, Batstone DJ (2020). Autotrophic sulfide removal by mixed culture purple phototrophic bacteria. Water Res. 182: 115896. DOI: 10.1016/j.watres.2020.115896 [45]

3.1 Introduction

Purple phototrophic bacteria (PPB) are a group of micro-organisms that grow anaerobically in the presence of infra-red light. From a waste treatment perspective, PPB technology has been explored as a technology that captures carbon, nitrogen (N), and phosphorus (P) in wastewater and converts these resources into a protein rich biomass product. PPB biomass is characterized by up to 65% protein on a dry weight basis [45], with potential applications as a either an organic fertilizer or as a feed additive/substitute in livestock, poultry and aquaculture applications.

However, many species of PPB can grow photo-autotrophically using sulphide as electron donor to reduce inorganic carbon for biomass growth [25, 55, 61, 84], making PPB an interesting candidate for an H_2S removal process with simultaneous stoichiometric CO_2 removal.

Raw biogas from piggeries contains H_2S at concentrations ranging from 700-4000 ppm (volume basis). H_2S is corrosive and toxic at these concentrations and requires removal from the gas before usage. Biological treatment processes using phototrophic microorganisms are a potential strategy for low-cost and scalable H_2S removal. Therefore, the aims of this component of the project were to:

1. Investigate a fully autotrophic process for sulphide removal via PPB to estimate sulphide removal rates as well as biomass yield.
2. Demonstrate PPB-based sulphide removal in a continuous process and identify important design parameters for a full-scale process.
3. Assess the viability of continuous PPB-based sulphide removal process, including comparison to existing desulfurization technologies.

3.2 Batch Experiments

3.2.1 PPB Culture

All batch tests were inoculated with a mixed PPB culture enriched from domestic wastewater and maintained at anaerobic conditions in modified Ormerod medium [22]. Acetic acid was added weekly as the carbon source and the culture was maintained with incandescent infra-red irradiation. The inoculum concentration was adjusted to 128 ±15 $mgCOD.L^{-1}$ for all experiments. The microbial community of the inoculum was not adapted to sulphide loading prior to the experiment.

3.2.2 Batch Apparatus

The experiments were conducted in 500 mL serum bottles (Wheaton, Illinois, USA) in triplicates, plus one dark control wrapped in aluminium foil to exclude any light input. The bottles were agitated in a temperature controlled MAXQ4000 Incubator Shaker (Thermo Fisher Scientific, Massachusetts, USA) with the temperature set at 24°C and the shaking speed at 200 rpm. The shaker hood was covered in aluminium foil to exclude any external light input from the environment. Inside the shaker, infra-red light emitting diode (IR-LED) spotlights (peak l=850 nm) were installed at two different distances from the serum bottles to create two different irradiation intensities. In order to quantify the intensity, the irradiance density emitted by the spotlights was measured with a radiometer (StellarNet, Tampa, Florida, USA) over a wavelength spectrum (l=300-1000 nm) at 21 grid points of the projected area of light incidence into the bottles. The obtained grid point values were interpolated cubically over the irradiated area to calculate the incident irradiance.

3.2.3 Reactor Inputs

A synthetic medium was used in two separate batch tests to obtain growth dynamics without micro- or macro-nutrient limitation for the quantification of sulphide removal. Experiments used a modified Ormerod medium [46] where all sulphate salts were replaced with chloride salts, in order not to interfere with sulphate measurements. The modified medium composition is presented in Egger 2020 [47].

The main substrates (i.e., sulphide as electron source, nitrogen, and inorganic carbon as carbon source) were added separately to the modified Ormerod medium:

- Sulphide was added as $Na_2S.9H_2O$ (equivalent to 150 $mgS.L^{-1}$) to ensure sulphide did not limit uptake rates
- Inorganic carbon was added as $NaHCO_3$ at 350 $mg.L^{-1}$ and 700 $mg.L^{-1}$ for low irradiance experiments and 960 mgL^{-1} for high irradiance experiments. These concentrations were selected to ensure that sulphide removal rate was not being limited by inorganic carbon availability.
- Nitrogen was added as NH_4Cl at 405 $mg.L^{-1}$ (equivalent to 105 $mgN.L^{-1}$), in all experiments with the synthetic medium.

Additional experiments were conducted using anaerobic digester centrate as the medium. Anaerobic digester centrate was collected from the centrifuge liquid return line of a local domestic wastewater treatment facility in South East

Queensland, Australia and contained inorganic carbon at 427±4 mg.L^{-1}; ammoniacal nitrogen at 503±28 mg.L^{-1}; and inorganic phosphate at 29±3 mg.L^{-1}. The centrate also contained total COD and soluble COD at 143±30 mgCOD.L^{-1} and 106±17 mgCOD.L^{-1}, respectively. All values are given with 95% confidence intervals. The centrate was diluted 50:50 with Milli-Q® water to lower the risk of microbial inhibition due to humic substances in the centrate. In the experiment using centrate as nutrient source, only the electron donor, sulphide was added as $Na_2S.9H_2O$ (equivalent to 150 mgS.L^{-1}). Iron (Fe) was added to supplement micro-nutrients (8.5 mgFe.L^{-1} added as $FeCl_2.4H_2O$).

3.2.4 Experimental Design

Experiments were conducted at 2 levels of irradiation:

1. For low irradiation experiments, the bottles were placed an approximate distance of 20 cm to the light source, the overall irradiance incidence on the bottle surface was 27 ± 3 W.m^{-2} and the irradiated area was 0.012 m^2. This irradiance was the same for two batch tests with synthetic medium and one test with digester centrate.

2. For high irradiation experiments, the bottles were placed an approximate distance of 3 cm to the light source, the overall irradiance incidence on the bottle surface was 56 ± 11 W.m^{-2} and the irradiated area was 0.012 m^2.

For all experiments the bottles were filled with the mixed medium, inoculum, and the carbon, electron and nitrogen source. The bottles were closed with a gas-tight rubber septum and the headspace sparged with nitrogen gas for five minutes to assure anaerobic conditions. Liquid samples were taken from all serum bottles, to quantify rates of sulphide removal, biomass growth and accumulation of by-products as well as consumption of macro-nutrients.

Samples were taken i) immediately after inoculation, ii) after an adaptation period of approximately 60 hours, iii) every 5 to 8 hours after adaptation until the concentration of sulphide was low, and iv) after 260 hours. Each sample was analysed for COD, sulphur species, dissolved inorganic carbon CO_2-C and the macro nutrients NH_4-N and PO_4-P. Elemental analysis was conducted for batch tests with digester centrate to determine the concentration of certain micro-nutrients (Fe, Mn) in the centrate, and thereby assess if the centrate was suitable as a medium for sulphide removal.

3.3 Continuous Experiments

3.3.1 PPB Culture

PPB biomass was originally enriched from domestic wastewater supplied with a synthetic nutrient medium [47]. The PPB biomass was then used in 2 experimental runs. During the first run, the biomass concentrations after inoculum were 103

mgCOD.L^{-1} in Reactor 1 (R1) and 68 mgCOD.L^{-1} in Reactor 2 (R2). A second run was inoculated from the residual biomass of the first run.

3.3.2 Continuous Reactors

Experiments were conducted in 2 L bubble column reactors set up and operated as a continuous process, labelled Reactor 1 (R1) and Reactor 2 (R2). The reactors were configured in an outdoor enclosure to mitigate risks associated with H_2S release. Figure 3 shows a schematic and image of the reactor set up. The reactor dimensions were H = 500 mm x D = 72 mm, wall thickness = 2 mm.

Components of the reactor system were:

1. Mass flow controllers (Bronkhorst, Netherlands) used to add gas at a set mass flow.
2. Gas diffuser ring (eight holes 1 mm diameter) used to distribute gas into the reactor liquid evenly.
3. Condensate trap: After passing through the reactor, the residue gas was cooled to remove most water vapour.
4. Gas flow meter (Bronkhorst, Netherlands): used to measure the mass flow of reactor off-gas.
5. H_2S sensors (Alpha sense, United Kingdom), to measure H_2S between 0-2000 ppmV in a bypass line to record periodic inline measurements.
6. H_2S destruction trap: Before venting the remaining gas to the environment, any remaining H_2S was destroyed in two sets of traps containing NaOH (2M) and FeCl (2M).
7. Online reactor pH and temperature sensors (Mettler Toledo, Ohio USA).
8. pH control system/pump system:
    a. The pH was controlled by pumping a NaOH solution (2M) into the reactor. The reactor volume was maintained by simultaneously removing the same volume of liquid introduced by the pH control.
    b. These pumps were later used as feed/effluent pumps to supply nutrients and control the reactor hydraulic retention time (HRT) at 4 days.
    c. The pumps were peristaltic pumps (Seko, UK) with influent flow rates of 1.28 mL.s^{-1} and 1.34 mL.s^{-1} into R1 and R2, respectively.
9. IR-LED lamps: To supply radiative energy (3 x 96 LED boards, λ=850 nm, Irradiance = 264.6 ± 56.9 W.m^{-2} at the incident, projected irradiated surface area of each column equal to 0.036 m^2).
10. A Process logic controller (PLC): Used to control the pump processes (i.e. pH control or HRT control), and the timing of downstream H_2S gas analysis and to log data at desired time intervals.
11. A liquid sample point was in the middle of the column at h = 250mm.

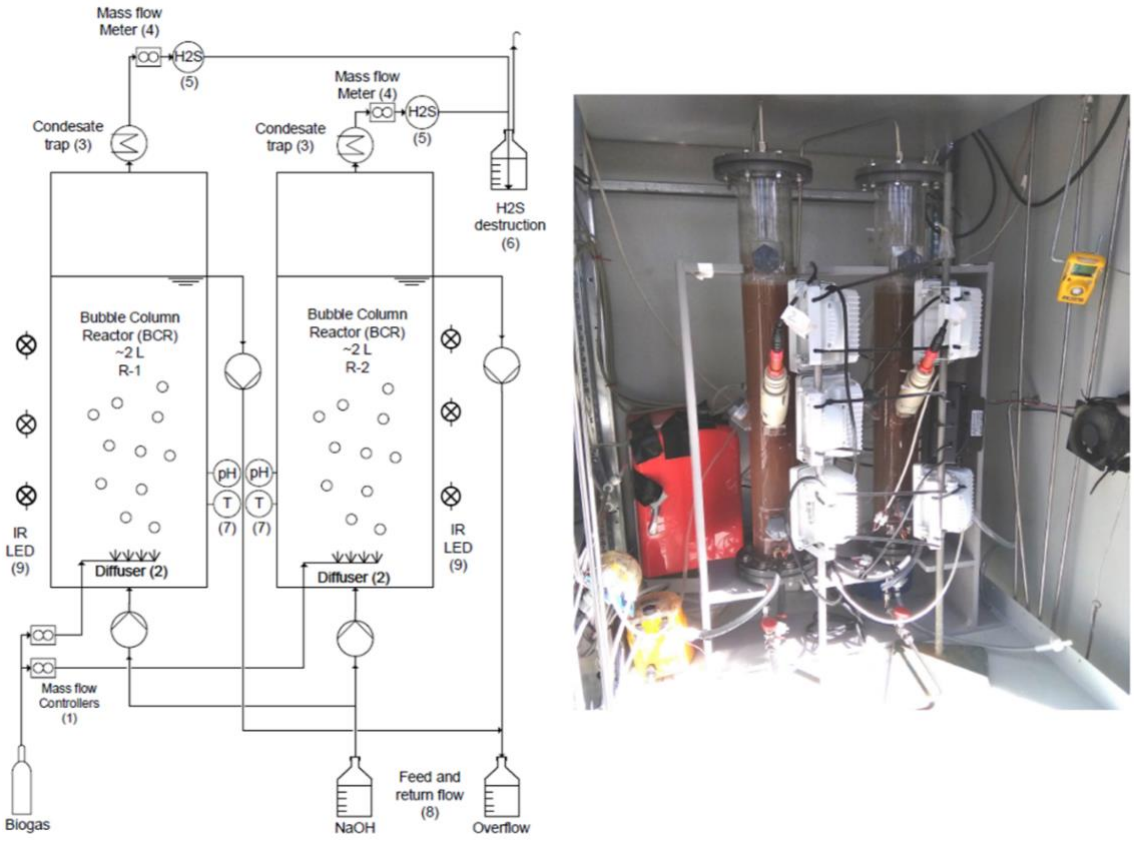

Figure 3 Schematic of the bubble reactor columns (left), photo of the reactors with PPB growing in the columns (right).

3.3.3 Reactor Inputs

The gaseous substrate was supplied as bottled biogas (BOC, Sydney Australia) with 30% CO_2, 0.2% H2S (2000 ppm, volume), and ~69.8% CH_4 (which resembles piggery biogas). The liquid medium in the reactor was diluted anaerobic digestion centrate obtained from a waste-water treatment facility in South-East-Queensland. The anaerobic digestion centrate was diluted 1:1 v/v with RO water and micro-nutrients were added to achieve the compositions shown in Table 8.

Table 8 Composition of medium for continuous reactor experiments using anaerobic digester centrate as the nutrient source

	Units	Reactor 1	Reactor 2
Total inorganic carbon (TIC)	$mgC.L^{-1}$	635.6	635.6
Ammonium nitrogen (TAN)	$mgN.L^{-1}$	393.5	393.5
Phosphate (PO_4-P)	$mgP.L^{-1}$	28.5	32.5
Organic acids (acetate)	$mg.L^{-1}$	71.8	68.1
Iron (Fe)	$mgFe.L^{-1}$	8.7	8.7
Manganese (Mn)	$mgMn.L^{-1}$	0.55	0.55

3.3.4 Mass Transfer Experiments

The H_2S gas-liquid mass transfer rate kLa (h^{-1}) was measured to compare physico-chemical rates to microbial kinetics. As both reactor columns are identical, only one column was used to determine the kLa. Two different gas flow rates (0.06 and 0.2 L min^{-1}) were trialled. The pH was controlled at 8.0 and this was considered during parameter estimation. The reactor liquid was reverse osmosis (RO) water pre-sparged with nitrogen to minimise dissolved oxygen and thus any sulphide oxidation. Liquid samples were drawn from the column's middle and immediately preserved for analysis. For 0.06 $L.min^{-1}$ biogas flow, an initial sample was taken before the gas flow was initiated, then every ten minutes for an hour, and three more samples every 30 minutes after that. For the higher biogas flow at 0.2 $L.min^{-1}$ the sampling frequency was increased accounting for a potentially higher gas-liquid mass transfer rate. Samples were taken every 2 minutes for 22 minutes after the initial sample, then three more samples at 25, 40 and 60 minutes. These samples rapidly preserved, and then analysed through ion-exchange chromatography (IEX) to determine the concentrations of dissolved sulphur compounds, specifically sulphide.

3.3.5 Continuous Reactor Operation

Experimental conditions for the continuous reactor experiments are shown in Table 9 and Table 10. Run 1 was inoculated with PPB enriched from domestic wastewater as previously described, and Run 2 was inoculated from the residual biomass of Run 1. For each run, the reactors were filled with 1.75 L medium and 0.25 L inoculum. During Run 1, the pH was controlled using caustic dosing. The pH was initially controlled at 6.9 and then increased to 7.6 (R1) or 7.8 (R2). For Run 2, the HRT was controlled at 4 days by dosing undiluted centrate medium into the reactors.

Three different gas flows were trialled, where 0.06 $L.min^{-1}$ biogas was established as the baseline. The corresponding sulphide loading rates were estimated at standard conditions (STP) with 2,000 ppm (Volume) H_2S in the biogas. The corresponding empty bed residence times (EBRTs) were 33.3 min (0.06 $L.min^{-1}$), 51.3 min (0.039 $L.min^{-1}$), and 16.7 min (16.7 $L.min^{-1}$), which are long in comparison to EBRTs in biofilter, bio-scrubber or trickling bed systems (10 s to 1 min) [48-50].

During continuous operation, the pH and temperature, the gas flow in and out of the reactors, and the downstream gas concentration were logged every 5 minutes, allowing for at least 8 data points in the off-gas H_2S per measuring period (40 min measuring following 40 min flushing with oxygen). Both reactors were sampled every 2 to 3 days for VFAs, COD, and dissolved sulphur species.

Table 9 Experimental conditions for Run 1. Run 1 was operated with variable HRT determined by NaOH dosing.

R1: Run	Operation time (h)	Biogas flow (L/min)	Empty bed residence time (min)	Sulphide load (STP) mg/L/h)	HRT (d)	NaOH dosing	Irradiance (W/inoculum/m2)	Inoculum
1.1	410	0.06	33.3	4.71	inf *	yes	264.6	WW
1.2	95	0.039	51.3	3.06	inf *	yes	264.6	-
1.3	68	0.039	51.3	3.06	inf *	no	264.6	-
2.1	171	0.06	33.3	4.71	4	no	264.6	Run 1
2.2	71	0.12	16.7	9.42	4	no	264.6	-

Table 10 Experimental conditions for Run 2. Run 2 was operated with controlled HRT of 4 days.

R2: Run	Operation time (h)	Biogas flow (L/min)	Empty bed residence time (min)	Sulphide load (STP) mg/L/h)	HRT (d)	NaOH dosing	Irradiance (W/inoculum/m2)	Inoculum
1.1	410	0.06	33.3	4.71	inf *	yes	264.6	WW
1.2	95	0.06	33.3	4.71	inf *	no	264.6	-
1.3	68	0.039	51.3	3.06	inf *	no	264.6	-
2.1	171	0.06	33.3	4.71	4	no	264.6	Run 1
2.2	71	0.12	16.7	9.42	4	no	264.6	-

3.4 Chemical Analysis methods

COD was determined according to standard methods 5220D [51] with a test kit (Merck Spectroquant (114541)) quantifying the total COD (tCOD) and soluble COD (sCOD). For the sCOD, samples were pre-filtered through a 0.45 µm filter (Merck Millipore, Darmstadt, Germany). Samples for the measurement of the soluble sulphur species (sulphide, sulphite, sulphate and thiosulphate), were filtered through a 0.22 µm filter and preserved with a Sulphide Antioxidant Buffer (SAOB) [52] using the method described by Pozo et al. (2016) [53]. These sulphur samples were analysed by ion-exchange chromatography with a Dionex ICS-2000 system equipped with a AG18 Dionex column (IEX) (Dionex, Sunnyvale, USA) and detected with a flame ionisation detector (FID) and spectrophotometrical measurement with a UV-VIS detector [53]. For the measurement of dissolved inorganic carbon (DIC) concentrations, samples were filtered through a 0.45 µm filter and analysed using a Shimadzu TOC-analyser (Shimadzu, Kyoto, Japan) [53]. Dissolved ammoniacal nitrogen and phosphate concentrations in the medium were measured with a Lachat QuickChem800 flow injection analyser (FIA) (Lachat Instrument, Milwaukee, Wisconsin, USA) after filtering the sample through a 0.45 µm filter. Elemental analysis was conducted on a filtered (0.45 µm) and non-filtered sample, after digestion with 10% nitric acid and using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer with Optima 7300 DV, Waltham, Massachusetts, USA). The concentration of acetic acid was measured through gas chromatography with a flame ionisation detector (Agilent Technologies 7890A GC System). Measurements of inorganic N and P, elemental analysis and acetic acid analysis were conducted according to the methods described by Hulsen et al. (2014) [22].

3.5 Microbial Community Analysis Methods

Microbial composition was analysed via genomic sequencing by the Australian Centre for Ecogenomics (ACE). DNA extraction and amplification was conducted by ACE. The universal primer pair Univ SSU 926F-1392wR was used, targeting regions of the 16S and 18S rRNA genes. Sequencing was conducted using the Illumina® platform. Reads identified as a single read, with relative abundance of less than 0.05 % or sequence identity less than 60% were discarded. 5198 operational taxonomic units (OTUs) were identified. Sequences were aligned using BLAST [54]. The resulting OTU table was analysed in R using ampvis2 [55].

3.6 Results

3.6.1 Batch Sulphide Removal

Figure 4 shows sulphide concentrations in the batch experiments using synthetic medium over time. Initially the sulphide was removed at a lower rate due to an adaptation period of the microbial community. After about 60 hours, the removal of sulphide became considerably faster and all sulphide was completely removed after 125 hours in both experiments.

In the experiment with higher irradiation, complete sulphide removal was achieved about 24 hours earlier than in the experiment with lower irradiation, indicating that the irradiation intensity affects reaction rate. In both experiments, a black precipitate was observed at the beginning of the experiment, indicating formation of iron sulphide. Sulphide was not removed in the dark control experiments.

The average volumetric sulphide removal rate in the low irradiance experiment was 1.79±0.16 $mgS.L^{-1}.h^{-1}$. The average volumetric sulphide removal rate in the high irradiance experiment was 2.9 $mgS.L^{-1}.h^{-1}$. Inorganic carbon was also removed during the experiments at close to the expected ratio of 0.5 mole S/mole C.

Sulphide and inorganic carbon removal caused the pH to increase from an initial 8.7 to 9.3 in the low irradiance experiment and an initial 8.5 to 9.0 in the high irradiance experiment. The high initial pH was caused by the addition of sodium sulphide. The subsequent pH increase was caused by the production of biomass, despite the formation of sulfuric acid. Residual acetic acid was low at the start of all experiments with synthetic medium, with the low irradiance experiment being less than 9.7 $mg.L^{-1}$ and less than 3.4 $mg.L^{-1}$ for the high irradiance experiment. For these low organic electron donor concentrations, the extent of photoheterotrophic growth was likely negligible.

Figure 5 shows sulphide concentrations in the batch experiments using synthetic medium over time. Like the synthetic media, sulphide removal in centrate was initially slow, and increased towards the end of the experiment. The average volumetric sulphide removal rate in the centrate experiment was 1.5±0.4 $mgS.L^{-1}.h^{-1}$. Inorganic carbon was also removed during the experiments, but at lower ratios than expected. As in the previous experiments, the pH progressively increased from 8.9 to 9.4.

Final sulphide removal rates using centrate were similar to synthetic medium. However, in centrate experiments the sulphide concentration increased towards the end of the experiment, indicating that sulphide removal was incomplete and was instead being re-released. It was hypothesized that re-release was due to incomplete enzymatic reactions as a consequence of low micro-nutrient concentrations of Mn and Fe, both in low concentration and both being a requirement for the enzymatic cascade of sulphide oxidation, which is dependent on Fe and Mn [56, 57].

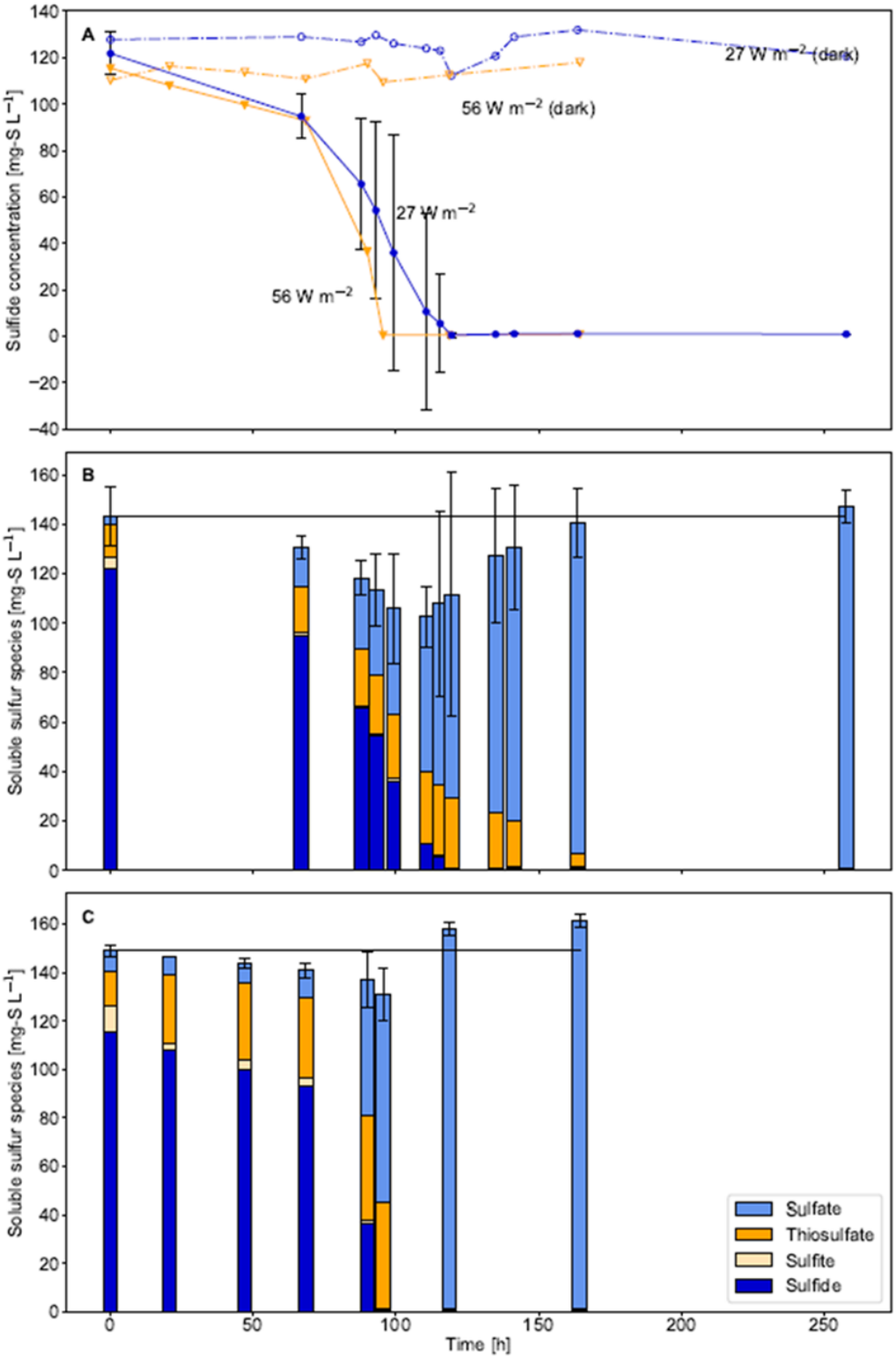

Figure 4: (A) Sulphide concentration in a low and high irradiance experiment showing increasing removal rates over the course of the experiment. (B) Balance of all soluble sulphur components (sulphide, sulphite, thiosulfate and sulphate) in the low irradiance experiment. (C) sulphur balance of the high irradiance experiment over time. Error bars indicate standard deviation of the balance. The horizontal, dashed line is an indication of mass balance closure during the experiment. Error bars are 95% confidence intervals in all figures. (Reproduced from [45])

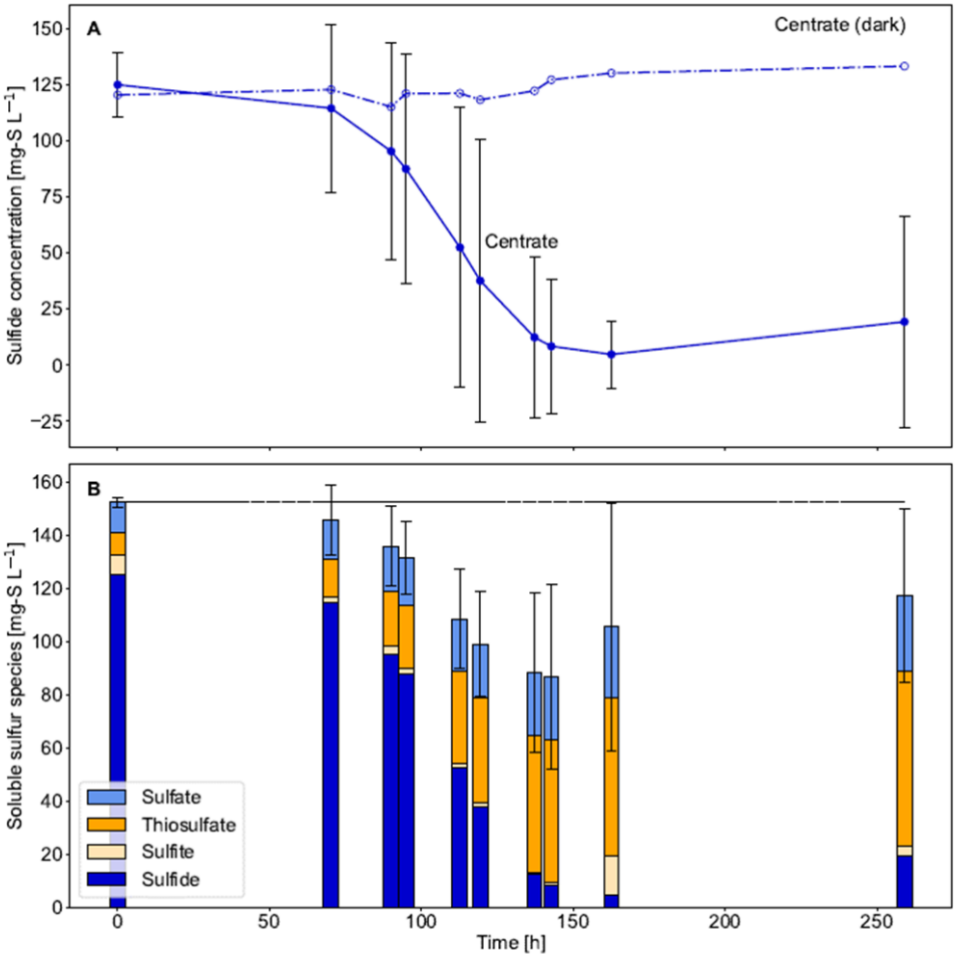

Figure 5: Sulphide removal using centrate as medium (A). Sulphur balance in (B) shows that sulphide removal was not complete with residual sulphide remaining at the end of the experiment and the oxidation chain seemingly interrupted by accumulating thiosulfate and intracellular sulphur. Error bars indicate 95% confidence intervals. (Reproduced from [45])

3.6.2 H_2S Mass Transfer for Continuous Process

The first critical step in all H_2S removal technologies is the transfer of H_2S from the gaseous phase into either a liquid phase (via absorption), or onto a solid carrier (via adsorption). In the case of biological processes, the H_2S is then oxidised through a series of biological reactions, and thereby removed. The initial mass transfer processes are described using a mass transfer coefficient kLa.

Gas-liquid mass transfer coefficients (kLa) were estimated by varying gas flow rates and measuring H_2S in the liquid phase, and results are presented in Figure 6. At a gas flow rate of 0.06 $L.min^{-1}$ the mass transfer coefficient was 0.55 ± 0.02 h^{-1} and at flow rate of 0.2 $L.min^{-1}$, the mass transfer coefficient was 2.06 ± 0.09 h^{-1}.

For a H_2S concentration of 2000 ppm (volume) in the feed gas and a gas flow rate of 0.06 $L.min^{-1}$, the expected maximum transfer rate of sulphide into the reactor is 3.5 $mgS.L^{-1}.h^{-1}$ (assuming no accumulated sulphide in the liquid phase); this increases to approximately 13.2 $mgS.L^{-1}.h^{-1}$ at a flow rate of 0.2 $L.min^{-1}$.

PPB biomass has been shown to remove sulphide at approximately 0.12 mgS per $mgCOD_{biomass}.h^{-1}$. Therefore, biomass concentrations of 30 $mgCOD.L^{-1}$ and 115 $mgCOD.L^{-1}$ would be sufficient to completely remove the dissolved sulphide at gas flow rates of 0.06 $L.h^{-1}$ and 0.20 $L.h^{-1}$ respectively (irradiance of 56 $W.m^{-2}$). Accordingly, at these sulphide supply rates, mass-transfer is limiting.

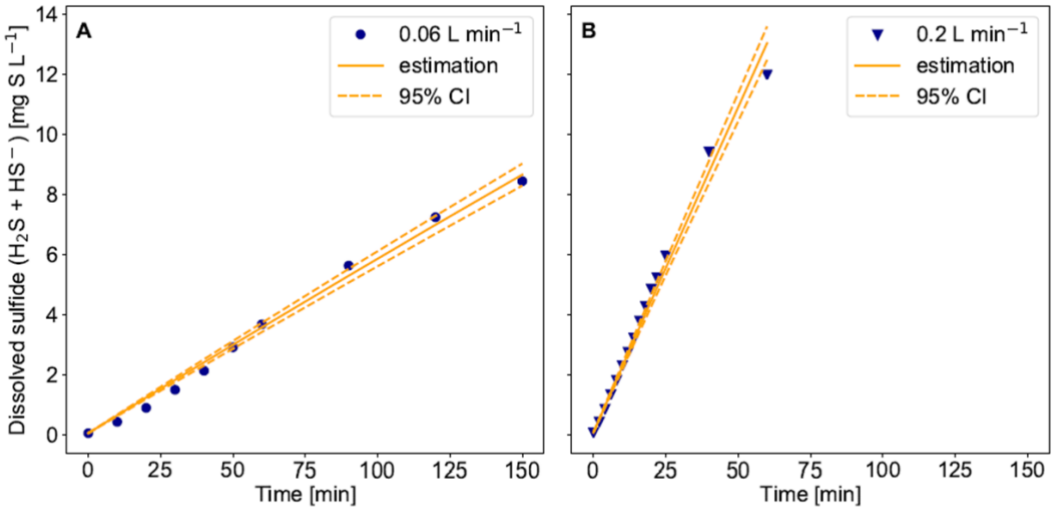

Figure 6 Gas-liquid mass transfer experiments for H_2S with (A) low gas flow at 0.06 L min^{-1} and (B) high gas flow at 0.2 L min^{-1}, at control pH 8.0 considered in the parameter estimation. Model estimation and confidence in the model are provided.

3.6.3 Continuous H_2S Removal

Results from the continuous reactor operation are summarised in **Figure 7**. During continuous experiment 1 (where the HRT was not controlled), the PPB desulfurization achieved in R1 was 89.02 ± 0.17% (maximum of 94.16%) and the PPB desulfurization achieved in R2 was 89.52 ± 0.16% (maximum of 96.70 %). The corresponding sulphide removal rates were 2.7 $mgS.L^{-1}.h^{-1}$ and 4.3 $mgS.L^{-1}.h^{-1}$. During continuous experiment 2, where the HRT was controlled at 4 days, the process removed up to 6.6 $mgS.L^{-1}.h^{-1}$ in R1 with a sulphide loading rate of 9.4 $mgS.L^{-1}.h^{-1}$, being equivalent to a 70% removal efficiency. The average residual H_2S concentration in the gas was 575 ppm. During experiment 2, the sulphide removal rates in R2 were lower, which may have been due to a shift in the microbial community resulting in sub-optimal conditions for PPB effecting the sulphide removal.

Figure 8 shows COD concentrations in the continuous reactors during experiment 1. An observed increase in COD concentration was an indication of biomass growth and accumulation in the reactors. The results show growth of both suspended biomass

and biofilms. During experimental run 1, the suspended biomass concentration in both reactors increased from approximately 250 to 500 mgCOD.L^{-1}. Total biomass concentrations were higher reaching 1 gCOD.L^{-1} in R1 and 1.7 gCOD.L^{-1} in R2.

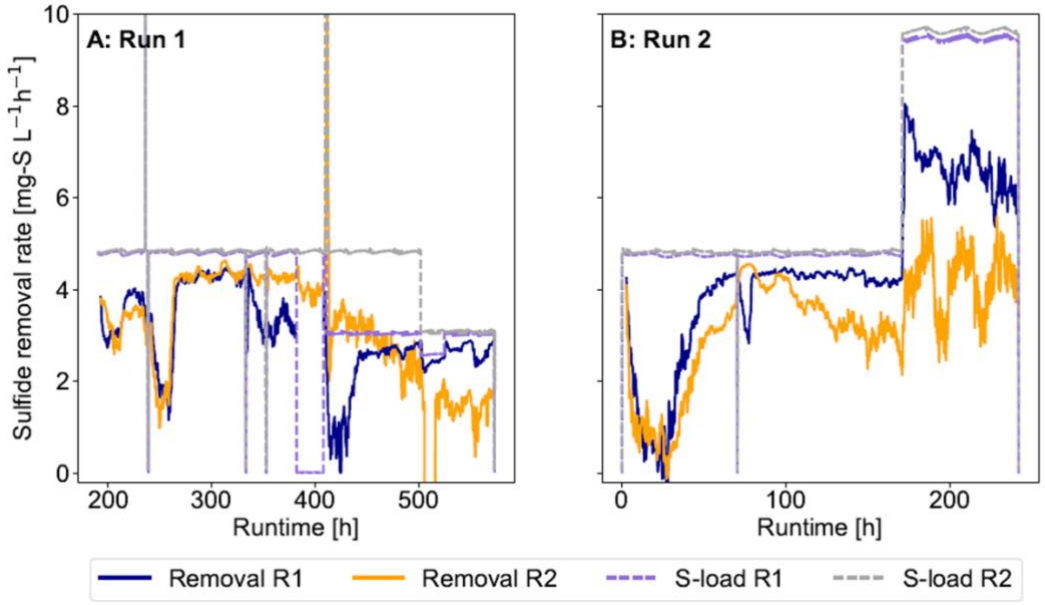

Figure 7 H_2S removal rates compared with sulphide loading rates (S-load) in Reactor 1 (R1) and Reactor 2 (R2) from 2000 ppmV H_2S in the biogas feed (A) Run 1 , (B) Run 2.

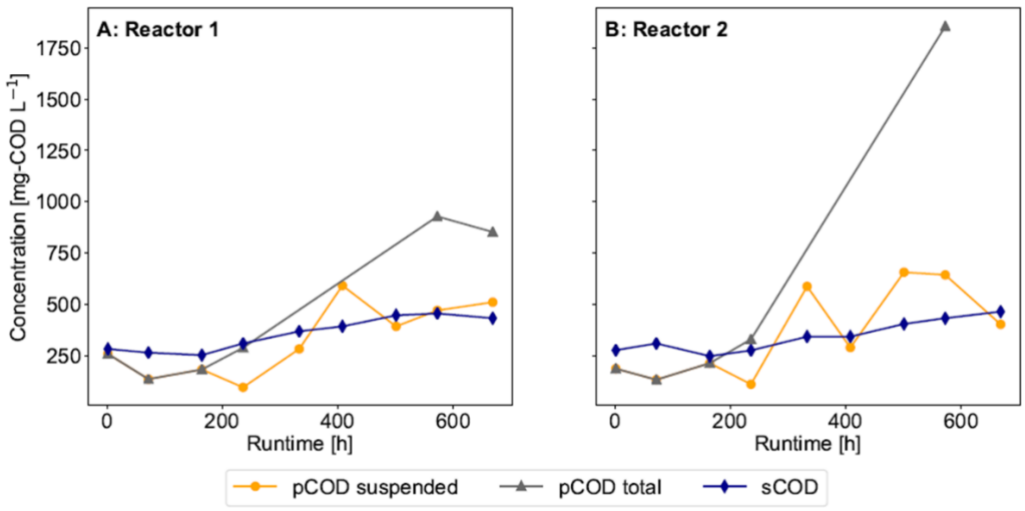

Figure 8 COD concentrations in (A) Reactor 1 and (B) Reactor 2 for experimental run 1. The biomass concentration is measured in particulate COD and the suspended and total (attached + suspended) concentration is presented here.

3.6.4 Regulation of pH

During biological desulfurization, pH decreases due to the conversion of sulphide to sulphate generating hydrogen ions. Figure 9 shows the pH in the continuous reactors during each experimental run. During run 1, the pH was initially controlled with caustic dosing. Once caustic dosing was stopped, the pH remained relatively stable due to carbonate buffering and residual alkalinity [11].

During run 2 the pH was not actively controlled. The pH results during run 2 show that the pH was relatively stable during the first 120 hours. There was limited biological activity and limited sulphide oxidation during this time. After 120 hours, biological activity and sulphide oxidation both increased and there was a progressive decrease in pH. Low pH is not a favoured operating condition. At pH below 7, equilibrium will increase the proportion of dissolved sulphide that is H_2S (pKa1 = 7) which could result in poor H_2S solubility and H_2S leaving the reactor unreacted in the gaseous phase. Additionally, the optimum pH for key microorganism expected to be in the process (e.g. A. vinosum) is 7 – 7.3 and a lower pH is potentially limiting to the biomass and sulphide oxidation rates [58].

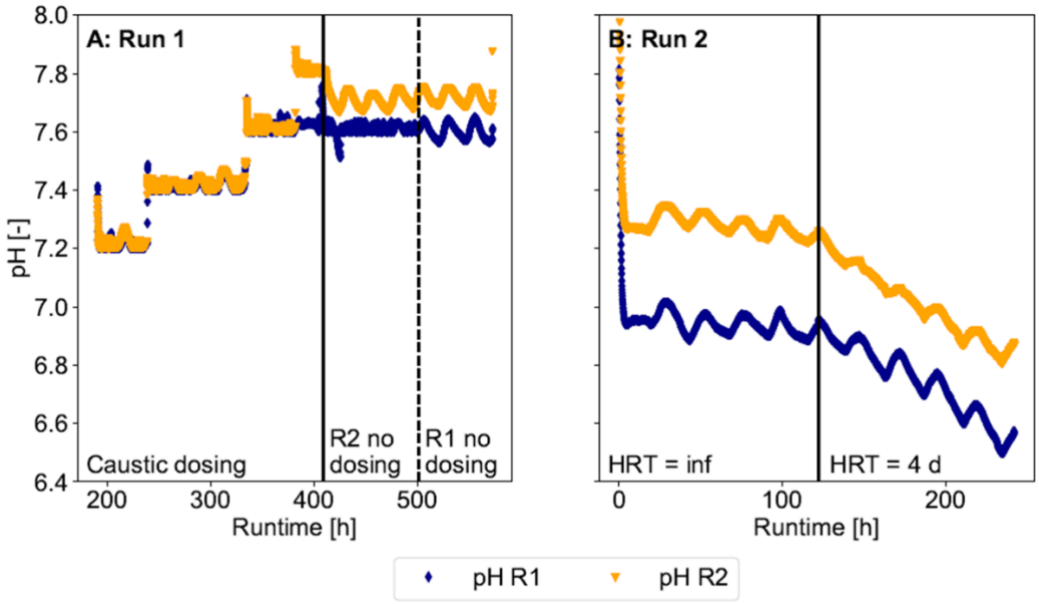

Figure 9 pH evolution during (A) run1 and (B) run 2 in reactor 1 (R1) and reactor 2 (R2). Caustic dosing phases and HRT control indicated for both runs. During run 1, the caustic dosing was stopped at 500 hours in reactor 1 and 410 hours in reactor 2.

3.6.5 Microbiology and Biofilm

Figure 10 shows the microbial communities in the reactors during experimental run 1. The results show that *Chromatiaceae*, the PPB family likely responsible for sulphide removal, was present in the inoculum in very low relative abundance (0.013 %) and increased significantly to 18.5% in R1 and 34.4% in R2. There was a relatively large flanking community of non-phototrophic organisms present in the reactor and

in the biofilms, and these flanking organisms may have been consuming decaying biomass as a carbon source. *Pseudomonadaceae* was the most significant non-phototrophic micro-organism in the reactor and was present in high amounts in the biofilms.

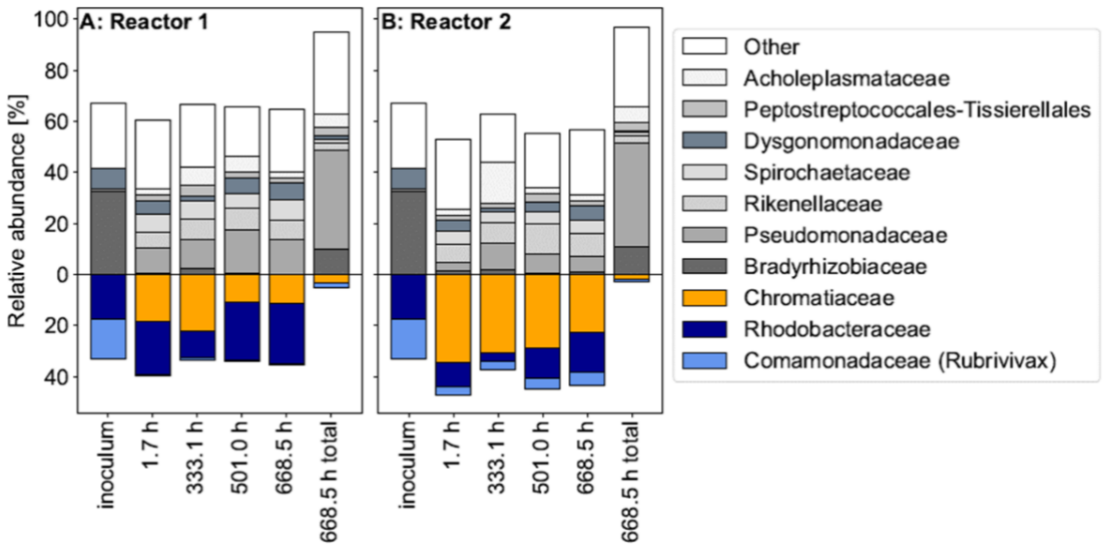

Figure 10: Relative abundance of the microbial community on the family level sand development with experimental progress for run 1 in (A) R1 and (B) R2. *Chromatiaceae* is the family of PSB effecting the sulphide removal. Note that the vertical scale only represents positive values.

At various stages in the experiments, the biofilms were gently scraped and resuspended into the liquid phase. Anaerobic resuspension of the biofilm did not impact on the removal performance, indicating that the biomass can remove sulphide equally effectively via either suspended or attached growth.

3.6.6 Model Based Process Analysis and Scale-up

Egger 2021 [11] developed a model capable of predicting H_2S removal and sulphate production in PPB desulphurization processes. This process model was used for a scale-up study. The scaled column diameter was 72 mm and the liquid height 6 m, resulting in a 25 L reactor with a gas headspace of roughly 5 L. An HRT of 2 and 4 days was implemented for the liquid flows and the results were compared to the experimental results for the 2 L continuous column reactor. The model simulation and experimental results are shown in Figure 11.

In the model simulations, sulphide loading rates up to 5 $mgS.L^{-1}.h^{-1}$ resulted in high sulphide removal rates and removal efficiencies of up to 78%, regardless of HRT. At higher sulphide loading rates of 10 $mgS.L^{-1}.h^{-1}$, the model showed a lowered pH at a HRT of 4 days resulting in biomass limitations and a decreased sulphide removal rate. Sulphide removal efficiencies decrease to approximately 57% under these conditions. This is partly addressed by decreasing the HRT from 4 to 2 days, which

maintains a higher pH and increased sulphide removal rates. Lower residual sulphide is potentially possible by optimising the process to a higher pH setpoint, e.g., by continued centrate addition at low HRTs.

At loading rates lower than to 5 $mgS.L^{-1}.h^{-1}$ there is near-complete oxidation of sulphide to sulphate. The residual H_2S in the gas is then dominated by saturation kinetics and mass transfer limitations. Gas-liquid mass transfer is independent of reactor volume in equivalent bubble column systems; similarly, control of HRT and pH also result in the same liquid concentrations in well mixed reactors. Therefore, the model predicts the same performance for both the 2L experimental reactors and a larger 25L scaled up reactors.

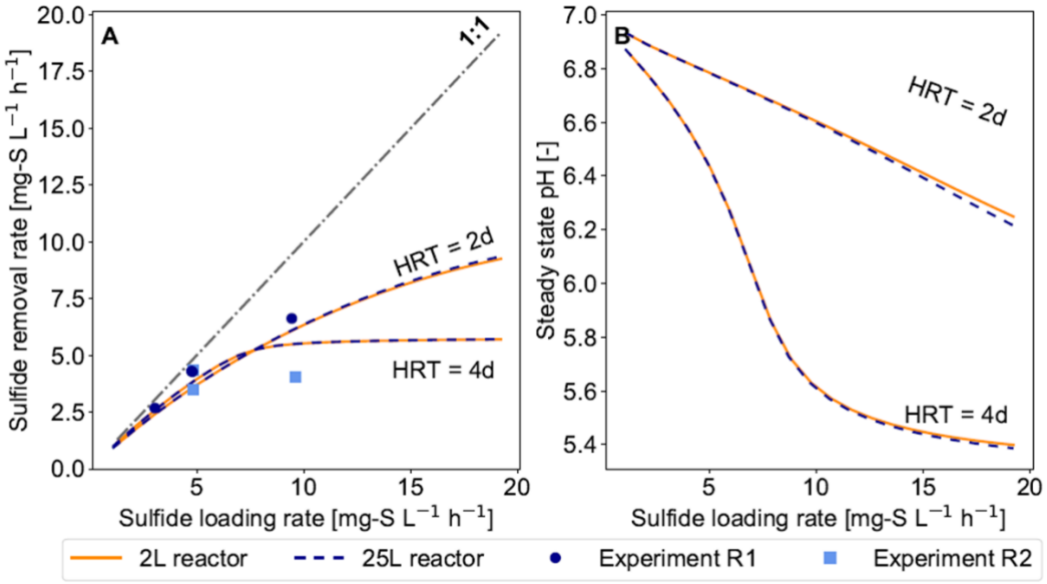

Figure 11 Continuous process model case study at laboratory and production scale for different sulphide loading rates in biogas containing ,2000 ppmV H_2S with a 2 day HRT and 25 L reactor volume; (A) sulphide removal rate vs sulphide loading rate, (grey dash-dotted line: sulphide loading = sulphide removal), blue dots are experimental sulphide removal rates from reactor 1, blue squares are experimental sulphide removal rates from reactor 2 (B) steady-state pH vs sulphide loading rate.

3.7 Application of Research

3.7.1 Comparison of PPB Driven Continuous Sulphide Removal to Other Technology

The PPB desulfurization process developed in this project removed H_2S from biogas with a maximum rate of 6.6 $mgS.L^{-1}.h^{-1}$. This is much lower than the sulphide removal rates achieved in competing technologies.

Other continuous phototrophic desulphurization systems, that remove sulphide from liquid and gaseous waste using green sulphur bacteria (GSB), reported a wide range of removal rates. The reported average sulphide removal from gaseous streams

using either pure culture *Chlorobium limicola* or mixed cultures is 62.7 ± 32.5 $mgS.L^{-1}.h^{-1}$ (error value is 95% confidence interval) [59-63], which is 9.5 times higher than the results achieved in this project. The maximum sulphide loading rates in the literature were 51.2 to 256 $mgS.h^{-1}$, compared to 18.5 $mgS.h^{-1}$ in this project. It is not clear if increasing the sulphide loading in this PPB desulphurisation process could also increase the removal rate.

Non-phototrophic sulphide removal systems that use sulphide oxidising bacteria (SOB) have average rates of 70 $mgS.L^{-1}.h^{-1}$ in biofilters and 90 $mgS.L^{-1}.h^{-1}$ in trickling beds, which again is much higher than the rates achieved in this project [12]. The most established biological desulphurisation process is the Thiopaq® process which is also an SOB driven sulphide removal system [64-66]. The volumetric rates for the Thiopaq® process have been estimated at up to 1500 $mgS.L^{-1}.h^{-1}$ [64], about 220 times larger than the rate achieved in the current process. The PPB process was pH limited as the concentration of sulphate increased. Higher removal rates may be possible by controlling the HRT and removing the accumulating sulphate; however, it is unlikely that a PPB process could achieve the sulphide removal rates of the Thiopaq® process.

A common H_2S removal system coupled to anaerobic digestion at wastewater treatment plants is adsorption with an iron sponge media [67, 68]. Commercial iron oxide pellets was found to completely remove 2000 ppm of H_2S for 177 hours with a rate of 809 $mgS.kg_{medium}^{-1}.h^{-1}$ before H_2S breakthrough was detected, and regeneration of the media was required after 500 hours [68]. Again, the PPB process achieved ~100 times lower sulphide loading than the commercial iron oxide media; but the PPB process does not require regeneration as does iron oxide media. This however does not compensate for the difference between PPB and iron sponge process capacities.

While the PPB process has not been optimised and process rats could be improved through management of pH, HRT and biomass, the current process loading rates are significantly lower than alternative technologies. The primary impact of lower loading rates is the need for larger PPB reactors compared to alternative technologies. However, as presented in the case study (Section 3.7.3), the PPB desulfurization reactors are relatively small compared to existing effluent treatment infrastructure at Australian piggeries (i.e., uncovered anaerobic ponds).

3.7.2 Chemical Free Desulphurisation

The continuous experiments were able to operate without caustic dosing and achieve stable pH. This was achieved due to either background carbonate alkalinity or using HRT (2 days) to continuously remove sulphate. Thus, PPB desulphurisation can operate without external chemical dosing for pH stabilisation, eliminating a large amount of the operating costs of conventional biogas scrubbers, such as those that operate with a caustic absorber. Chemical-free desulphurisation could be applied to remove up to 90% H_2S depending on the sulphide loading rate. Where higher levels of desulphurisation are required, for example to meet requirements for injection into natural gas grids, chemicals could also be dosed or could be used as a secondary polishing step.

3.7.3 Economic Evaluation of a Scaled Application

Table 11 shows an economic evaluation of an example PPB driven desulphurisation process sized for a 500 SPU piggery; the evaluation estimates operating costs but does not consider the capital costs of process equipment which were unknown due to the small scale of the current testing. Key observations from the economic assessment included:

- While the sulphide loading rates for the PPB process were an order of magnitude (or more) below the loading rates of some competing technologies, the reactor volume requirements for a 500 SPU piggery are less than $1m^3$; therefore, the PPB process is still a highly compact technology.

- The PPB process costs approximately \$85 kgS^{-1}. The major cost for the PPB process is the electricity used to irradiate the reactor overnight (estimated at $12h.d^{-1}$; but this would be lower in some areas, and/or could be supplemented using excess electricity generated from biogas).

- The PPB process does not require chemicals and therefore there are no chemical costs, this is a key advantage compared to competing technologies.

- The generation of protein-rich biomass is generally considered a key advantage of PPB technologies; however, the design and operating parameters from this project suggest that revenue from the PPB biomass accounts for as little as approx. 1% of the annual operating costs and is therefore not a significant factor.

Iron oxide media desulphurisation is a common and relatively low cost technology currently used to remove H_2S from biogas [67]. Costs for three different iron oxide media processes (SulfaTreat® , Sulfur-Rite® , Media-G2®) were estimated at \$9.20 US, \$5.2 US and \$3.2 US respectively [67]. Note, the reference plant was much larger and there may have been some economies of scale; regardless the iron oxide media costs are an order of magnitude lower than the PPB process.

Other biological processes for H_2S removal are estimated with reasonably low operational costs (Biofilter operational cost: € 0.2-0.3 EUR $(1000\ m^3)^{-1}$, bio-scrubber operational cost: €1-2 EUR $(1000\ m^3)^{-1}$, bio-trickling filter operational cost: €2-5 EUR $(1000\ m^3)^{-1}$ [12]. In all cases, the technologies are considerably cheaper than the PPB process.

Thiopaq® is a commercial desulphurisation process based on sulphate oxidising bacteria (SOBs), the major operating costs for the Thiopaq® process are aeration costs. The aeration requirements are 0.5 $kgO_2.kgS^{-1}$ which corresponds to an energy demand of 0.5 $kWh.kgS^{-1}$ [69]. Thus the aeration costs for Thiopaq® are estimated at \$0.05.kgS^{-1} [64, 65].

Table 11 Economic evaluation of an example PPB-driven desulphurisation process for a 500 SPU piggery.

Reactor Parameters from Project	**Unit**	**PPB Process**
Reactor Volume	L	25
Incident Reactor Area	m^2	0.432
HRT	d	2
Sulphide loading rate	$mgS.L^{-1}.h^{-1}$	10.62
H_2S concentration on the biogas	ppm	2,000
Residual H_2S concentration	ppm	751
Biomass concentration in Reactor	$mgCOD.L^{-1}$	529.5
500 SPU Case Study Inputs		
Annual biogas flow	$m^3.SPU^{-1}.y^{-1}$	50
Annual biogas flow	$m^3.y^{-1}$	25,000
Daily biogas flow (summer)	$m^3.d^{-1}$	82
Daily Sulphide Loading	$gS.d^{-1}$	215
Daily Sulphide Loading	$mgS.h^{-1}$	8,950
500 SPU Case Study Sizing and Performance		
Total volume	L	840
Total incidental area	m^2	14.5
Costs		
Electricity costs	$\$.kWh^{-1}$	0.10
Average Irradiation time	$h.d^{-1}$	12
Irradiation power demand	$kW.m^{-2}$	0.96
Total Power Demand	kW	13.5
Total Irradiation Electricity per year	$\$.yr^{-1}$	$5,928
Irradiation Electricity per kg S	$\$.kgS^{-1}$	$75.5
Compressor energy demand per kgS	$kWh.kgS^{-1}$	105
Compressor size	kW	0.94
Total Compressor Electricity per year	$\$.yr^{-1}$	$823
Compressor Electricity per kg S	$\$.kgS^{-1}$	$10.50
Revenue		
Protein Yield	$kg.kgS^{-1}$	0.587
Protein Production rate	$kg.d^{-1}$	0.126
Protein sales value	$\$.kg^{-1}$	1.1
Total Protein Revenue per year	$\$.yr^{-1}$	$50.7
Protein Revenue per kg S	$\$.kgS^{-1}$	$0.65
Net Cost		
Net Cost per year	$\$.yr^{-1}$	$6,700
Net Cost per kg S	$\$.kgS^{-1}$	$85.4

The PPB desulphurization process tested in this project is preliminary and has not been optimised. The process operates at a much lower biomass concentration than other conventional PPB technology developed specifically for production of microbial protein from waste streams. However, the irradiation requirements are not similarly reduced. The low biomass concentration and limited biomass production (due to S mass transfer limitations), combined with high irradiation intensity, result in prohibitively high operating costs. The PPB technology presented

in this project represents a proof-of-concept, rather than an optimised process. However, it is clear from the economic assessments that a PPB desulphurisation process would only be competitive if illumination costs are eliminated. Theoretically, a PPB process could operate with intermittent illumination (i.e., exposure to sunlight during daylight hours); however, different metabolic processes will likely occur during the dark periods. Intermittent illumination was not tested as part of this project. Alternatively, it was noted earlier in this report that excess biogas energy is often available at piggeries, which could be converted to electricity to utilise for illumination of a PPB process.

4 Upgrading Biogas to Biomethane using Micro-Algae

The work in this section was completed by Clemens Herold, Tasneema Ishika, Emeka G. Nwoba, Stephan Tait, Andrew Ward and Navid R Moheimani, led by Murdoch University and resulting in the publication:

Herold, C., Ishika, T., Nwoba, E. G., Tait, S., Ward, A., & Moheimani, N. R. (2021). Biomass production of marine microalga Tetraselmis suecica using biogas and wastewater as nutrients. Biomass and Bioenergy, 145, 105945. [70]

4.1 Introduction

Raw biogas from piggeries contains CO_2 at concentrations in the range 20-40% (volume basis). These gases need to be first cleaned to achieve a CH_4 content typically >90% [28] to be used as vehicle fuel or to be injected into a natural gas grid for supply of domestic and industrial markets.

Photosynthetic growth of microalgae is well documented as one of the most economical ways to sequester CO_2 [29], fixing approximately 1.83 kg of CO_2 to generate 1 kg of dry algal biomass [30]. As microalgae require significant amounts of CO_2 for growth, there is potential to use biogas as the CO_2 supply [33]. Additionally, microalgae can use piggery effluent (after anaerobic pond treatment) as a source of nitrogen and phosphorous for growth. Algal biomass typically has high growth productivity, high oil content and potentially high protein content, and therefore represents a value-add by-product [43].

One limitation of micro-algae technologies is the potential for high freshwater usage. Saltwater-based microalgae production systems can potentially reduce freshwater requirements by up to 90% [44]. Nutrients are a potential high cost of microalgae technologies that can be mitigated using effluent/wastewater as a source of nitrogen and phosphorus. *Tetraselmis sp.* is a marine microalgae that has been identified as an attractive candidate for an integrated biogas and effluent treatment system. *Tetraselmis sp.* has been found to endure high concentrations of CO_2 and can be cultivated using wastewater nutrients [41, 42].

Uptake of CO_2 from biogas using microalgal treatment is reported to be positively correlated to CO_2 dissolution rate [71]. CO_2 dissolution rate is dependent on the partial pressure of CO_2 in the biogas (pCO_2) and pH of the growth medium.

Higher partial pressure and higher pH improve reactive CO_2 mass transfer [71]. However, different microalgal species would also have an optimum pH for biomass growth and productivity [72]. Therefore, it is expected that an optimum pH would exist that both encourages CO_2 dissolution, and enables microalgae to utilize a higher percentage of CO_2 supplied in biogas to produce biomass. Therefore, the aims of this component of the project were to:

1. Integrate effluent treatment with biogas purification using a saline microalga, which has not been done before.

2. Determine the growth characteristics, biomass composition and maximum quantum yields marine microalga *Tetraselmis suecica* using synthetic biogas as a source of CO2, and ADPE as a source of nutrients.
3. Explore the impact of pH and the resulting CO2 partial pressure on microalgae growth
4. Assess the effect of CH_4 in biogas on microalgal cultivation of *Tetraselmis sp*.

4.2 Materials and Methods

4.2.1 Microalgae and Culture Media

The marine microalga *Tetraselmis suecica* (CS187) used for this study was obtained from the culture collection of CSIRO, Western Australia (WA). *T. suecica* was cultivated at natural seawater conditions (35% salinity). The seawater was collected from Hillarys Beach, WA and was charcoal filtered before use [73]. Piggery effluent after anaerobic digestion (ADPE) was used as a source of nutrients and was obtained from an anaerobic pond of a piggery located about 100 km north of Perth WA, and was sand filtered prior to use [74]. The ADPE was analysed to determine total nitrogen, phosphorus and trace metals. (Table 12). The ADPE was then added to seawater to achieve a medium with the same total nitrogen content of F media (as described by Guillard [75]), which resulted in the media containing approximately 20mL of ADPE per L media and a TN concentration of 24.7 $mg.L^{-1}$. The media was also enriched with vitamin solutions to align with F media composition as described by Guillard [75].

Table 12 Nutrient concentration of ADPE and approximate concentrations of Seawater + ADPE medium

Substance	ADPE Concentration [mg/L]	Seawater + ADPE Concentration [mg/L]
Total Ammonical Nitrogen	1,180	~23.1
NO_3-N	10	~0.2
NO_3^{2-}	20	~0.4
NO_2^-	230	~4.5
NO_2-N	70	~1.4
$NaNO_2$	340	~6.7
Total N	1,260	~24.7
PO_4^{3-}	100	~1.96
P_2O_5	70	~1.4
Total P	30	~0.6
$CaCO_3$	3,500	
Fe	13.8	
Ca	147	
Mg	151.2	
pH	8.063	

4.2.2 Experimental Setup

The experiment tested the effect of different pH set points and associated pCO_2 on biomass, lipid and carbohydrate productivity, chlorophyll a content, and light adapted maximum quantum yield. The CO_2 and nutrients consumption efficiency of *T. suecica* at different pH set points were also investigated. Additionally, the effect of CH_4 on the growth of *T. suecica* was tested.

The experimental set up is shown in Figure 12. During the experiment, *T. suecica* was cultivated in 2 L Erlenmeyer flasks with a working culture volume of 1.5 L. The culture was grown at temperature 25 ± 1 °C and a 12 h:12 h light/dark period was used. Light irradiance was 175 ± 25 µmol photons $m^{-2}.s^{-1}$ and was measured using a Li-185B quantum meter equipped with a PAR quantum sensor, Li-190SB. This irradiance was based on *T. suecica* Pmax [76]. Cultures were mixed using 40 mm magnetic stirrers set at 150 rpm. The cultures were operated in a batch mode and each treatment had four individual replicates (n = 4) with an experimental duration of 8 days.

During experiments a pH probe (Figure 12 - d) was placed inside the culture flask to determine the pH of the culture media. The pH probe was connected to a miniCHEM-pH Process Monitor (i). If the pH of the culture media increased to above the pH set point, the controller switched the solenoid valve (k), allowing the test gas to flow to the culture flask. The synthetic test gas flow rate was maintained at 100 $mL.h^{-1}$ using a gas flow controller (a). When the pH of the culture media decreased to below the pH set point by dissolving CO_2 from the test gas, the solenoid valve turned off the gas flow. Four treatment pH set points of 6.5, 7.5, 8.5 and 9.5 were tested for the synthetic gas with N_2 and one pH set point of 7.5 was tested for the synthetic gas with CH_4.

Experiments were conducted with synthetic test gases from BOC, Australia. The majority of experiments used synthetic gas containing 40% CO_2 and 60% N_2. The CO_2 content of this mixture mimics typical piggery biogas concentrations. N_2 was used as a substitute for CH_4 to eliminate the risk of forming flammable gas mixtures. A smaller subset of experiments used a synthetic gas mixture comprised of 80% CH_4 and 20% CO_2 (BOC, Australia) to test potential impacts of dissolved CH_4 on microalgal growth performance. This mixture was selected to keep the experimental conditions above the upper flammability limit for CH_4 in pure oxygen [77]. The experiments were performed in a fume hood to prevent any possibility of CH_4 accumulation. An uncontrolled pH (negative control) was also tested using only atmospheric air supplied continuously with no additional CO_2. For this, an air pump was used to generate an atmospheric air flow rate of 100 $mL.h^{-1}$ through the control culture.

To ensure consistency, microalgal culture samples were collected at 12:30 pm on each sample day. The sampling was undertaken on days 1, 4, 6 and 8. Samples were analysed to determine biomass yield (both dry weight and organic weight), cell density, light-adapted maximum quantum yield (Fq'/Fm') and culture media alkalinity. The lipid, carbohydrate, total nitrogen, phosphorus and chlorophyll a contents were measured on days 1, 4 and 8.

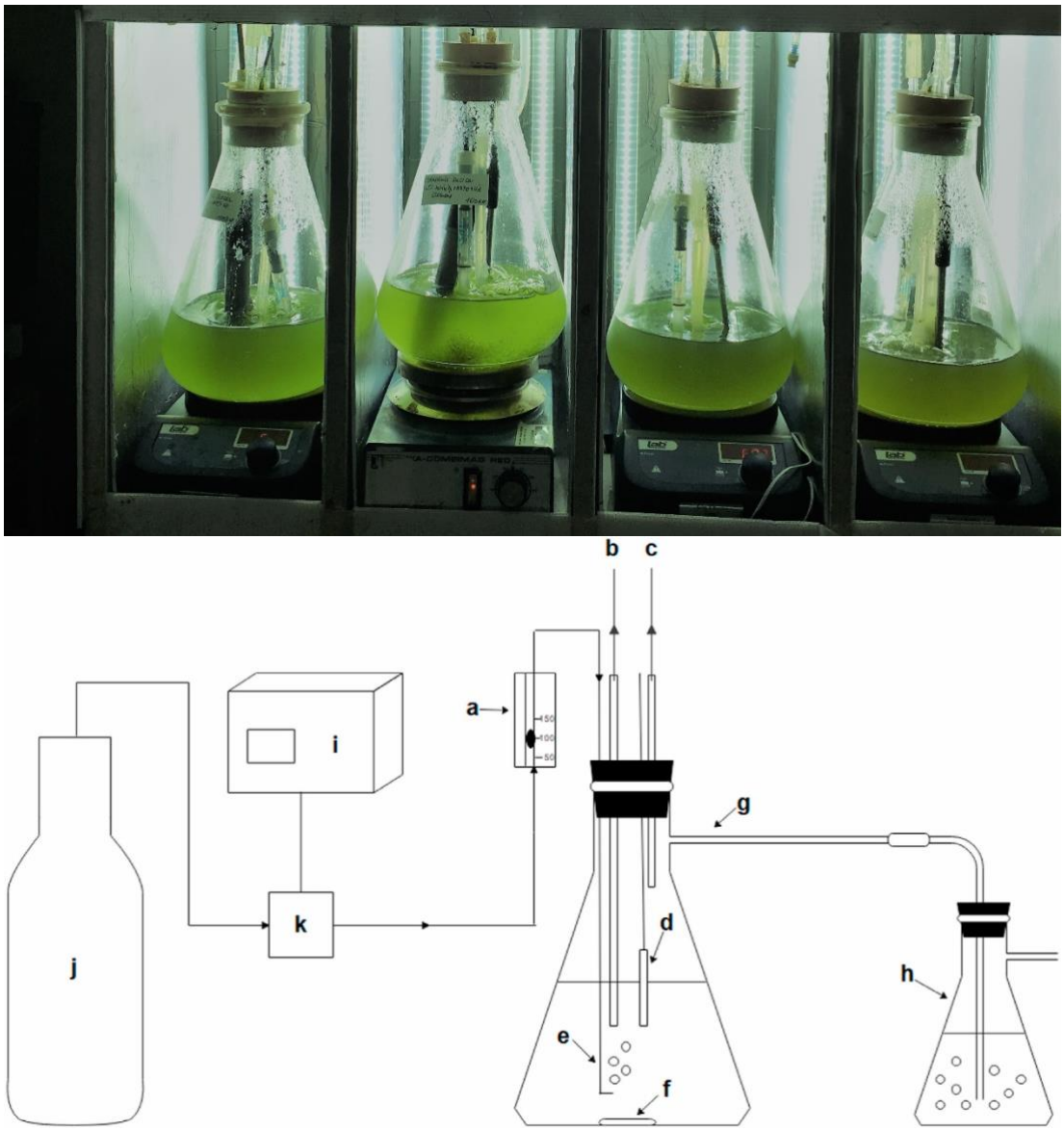

Figure 12: Experimental setup: a - flow meter (mL h^{-1}), b - one way tube for culture sampling, c - one way tube for gas sampling, d - pH probe, e - gas diffuser, f - magnetic stirrer, i - pH controller, j - pressurised gas cylinder containing synthetic test gas, k - solenoid valve. (Reproduced from [70])

4.2.3 Analytical Methods

Samples for measuring microalgae biomass weight, lipid, carbohydrate and chlorophyll a content were filtered using Whatman 2.5 cm GF-C filters. The filtered microalgae samples were then rinsed with isotonic ammonium formate solution to remove residual salt [78]. Fresh filtered samples were used to measure the biomass weight, while samples for measuring lipid, carbohydrate and chlorophyll a content were stored at −80 °C until analysed. Fresh microalgae culture samples were used to measure cell density and Fq'/Fm'. Before measuring total nitrogen, phosphorus content and alkalinity of the culture media, microalgal biomass was removed from the culture media by centrifugation [79].

The dry weight (DW), organic weight (ash-free dry weight, AFDW) and biomass productivity were determined using the method described in Moheimani *et al.* [80]. Microalgal cell density was determined with the Improved Neubauer cell counting chamber and light microscope. Lipid extraction was performed using the Bligh and Dyer method as modified by Kates and adapted by Mercz [81-83]. The lipid content was expressed as % of organic weight. Lipid productivity was determined by multiplying lipid content with biomass productivity (organic weight) and expressed in units of milligram per L of culture medium per day. Total carbohydrate content was determined based on the method described by Kochert and Ben-Amotz *et al.* and modified by Mercz [83-85]. Carbohydrate content was expressed as % of organic weight. Carbohydrate productivity was determined by multiplying carbohydrate content with biomass productivity (organic weight), and the value was expressed per L of culture medium per day. The method of Jeffrey and Humphrey was used to determine chlorophyll *a* content [86] and was measured using a spectrophotometer at 664 nm and 647 nm wavelengths. The whole extraction process was carried out under dim light to prevent degradation of chlorophyll *a* pigment.

To determine alkalinity, total nitrogen, total phosphorus, iron and COD content of the filtered culture media, a Multiparameter Photometer HI 83099 (Hanna Instrument, USA) was used with various Hanna test kits (Table S4) in accordance with manufacturer's protocols. Magnesium and calcium content in ADPE were determined by Spectroquant® Move 100 (Merck KGaA, Germany) using test kits in accordance with manufacturer's protocols. The partial pressure of CO_2 (pCO_2) in cultures was determined from temperature, salinity, phosphate, total alkalinity and pH of the medium using the CO_2sys software (v2.1) and the constants of Roy *et al.* for seawater [87].

To determine the organic carbon content, the culture was first centrifuged for 15 min at 4000 rpm, and pellets dried at 60 °C for 24 hours. Dry biomass was ground finely with a mortar and pestle and then stored at -20 °C in the freezer until analysis. 1.5 – 2 mg of ground biomass was weighed and the organic carbon content was analysed using a Perkin Elmer 2400 Series II CHNS/O-Analyser [88]. The results were expressed as % of dry biomass.

4.2.4 Photosynthetic Measurements

The light-adapted maximum quantum yield (F_q'/F_m') is the ratio between variable and maximum quantum yields. F_q' shows the maximum variable fluorescence in light-adapted state, which shows the difference between F_m' *and* F_o' ($F_q' = F_m' - F_o'$), where F_o' is the minimum fluorescence intensity with all reaction centres of PSII open in the light-adapted phase and F_m' the maximum fluorescence intensity with all reaction centres of PSII closed in the light-adapted phase [89]. On day 6 of each experiment, the F_q'/F_m' was measured diurnally, i.e. at 05:30 am (30 min before the light switched on), 06:30, 09:30, 12:30, 15:30 and at 18:30 (30 min after the light switched off) [90]. In the subset of experiments with synthetic gas containing CH_4, biomass productivity and effective quantum yield of PSII primary photochemistry (F_q'/F_m') were measured to evaluate the growth potential of the algal cells, with biomass density determined daily, and F_q'/F_m' values also measured daily before illumination (06:00) and at 30 minutes before the start of the dark regime (17:30).

Light-adapted maximum quantum yield measurements were performed using a hand-held fluorometer AquaPen AP 100-C (Photon Systems Instruments, CZE) paired with the FluorPen 1.1 software. This instrument is equipped with a high-intensity LED-array, which can emit red light (620 nm). During the measurement, a high-pulse light (3000 µmol photon m^{-2} s^{-1}) irradiates for less than a second to stimulate the photosystems of the sample. The emitted fluorescence from the sample is then recorded by the AquaPen.

4.2.5 CO_2 Capture Efficiency of *T. suecica* Cultures at Different pH Set-points

Gas samples were taken from the culture headspace immediately after each gas-switching off cycle on days 1, 4, and 8. The gas samples were withdrawn using an air-tight Luer-Lock gas syringe and were analysed using a gas chromatograph to determine the residual concentration of CO_2 in the outflow gas. CO_2 concentrations in the influent and outflow gas were analysed using an Agilent 7820A gas chromatograph (GC) with flame ionisation detector (FID). A total sample volume of 100 µL was injected directly onto an Altech Econo-CapTM ECTM-1000 column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). The carrier gas was N_2, set at a flow rate of 3 $mL.min^{-1}$. The oven temperature was programmed as follows: initial temperature 70 °C, increased at 5 °$C.min^{-1}$ to 100 °C, held for 2.0 min, increased at 70 °$C.min^{-1}$ to 250 °C, held for 2.0 min. Injector and detector were set at 250 and 300 °C, respectively. The peak area of the FID output signal was computed via integration using the EzChrome Elite Compact Software© (V.3.3.2 SP2) [91]. The CO_2 capture efficiency was calculated as [92]:

$$\text{CO}_2 \text{ capture efficiency } (\%) = \frac{(\text{CO}_2 \text{ in influent} - \text{CO}_2 \text{ in culture headspace})}{\text{CO}_2 \text{ in influent}} \times 100\%$$

4.3 Results

4.3.1 Effect of pH and Associated CO_2 Content on Algae Growth

Figure 13 shows the effect of pH set point on the biomass productivity, pCO_2, lipid and carbohydrate productivity, chlorophyll a content and light adapted maximum quantum yield (Fq'/Fm') in experiments without CH_4 in the test gas. The results show an overall increase in biomass productivity as the pH set point decreased. The highest overall biomass productivity of 59.8 $mg.L^{-1}.d^{-1}$ was found at the pH set point of 7.5. Biomass productivity was not significantly different between the pH 6.5 and pH 7.5 set points (One Way ANOVA, $P>0.05$). However, biomass productivity at the pH 7.5 was significantly higher (One Way ANOVA, $P<0.05$) than at the pH set points of 8.5, 9.5 and at the uncontrolled pH (the control test), respectively.

Overall, lipid and carbohydrate productivities appeared to mirror the decreasing trend of biomass productivity with increasing pH set point values. However, no significant differences were observed in lipid and carbohydrate productivity at the various pH set points (One Way ANOVA, $P>0.05$). Lipid content of the uncontrolled pH experiment (55.1% ± 4.2% of AFDW) was 56% higher than at the 8.5 and 9.5 pH set points. Carbohydrate content was significantly higher at the pH set points of 6.5

and 7.5 (13.3 ± 0.5% of AFDW) as compared to the other pH set point conditions and the uncontrolled pH test (One Way ANOVA, $P<0.05$).

A gradual increase in chlorophyll a content was observed with increasing pH, also supported by a Pearson's correlation (correlation coefficient 0.961, $P = 0.039$). The highest chlorophyll a content was found at pH 9.5 (1.5% ± 0.06% of AFDW), which was significantly higher than at the pH 6.5, 7.5 and uncontrolled pH treatments (One Way ANOVA, $P<0.05$) (Figure 13). The overall light adapted maximum quantum yield (Fq'/Fm') was greater than 0.6 at each of the pH set points. The Fq'/Fm' value was significantly higher at the pH 7.5 set point than under the uncontrolled pH conditions (One Way ANOVA, $P<0.05$) (Figure 13).

The light adapted maximum quantum yield (*Fq'/Fm'*) of *T. suecica* was measured over the day (during both light and dark photoperiods). An *Fq'/Fm'* value over 0.6 is generally used to represent 'healthy' cultures while values under 0.6 typically suggests some form of stress experienced by the algal cells [93]. With all pH set points, the *Fq'/Fm'* increased up to a maximum of 0.74 during light period and decreased to 0.68 during the dark period; indicating that the algae was not subjected to any significant stress. However, at the uncontrolled pH conditions (negative control), the Fq'/Fm' values were found to decrease gradually over the light period and further decreased to a low of 0.55 during the dark period. This indicates stress under conditions where pH isn't controlled. Algae grown under stressed conditions has reduced photosynthetic rate [94], and this resulted in lower overall biomass productivity. The results demonstrated that pH control may be important to maximise biomass growth/productivity and therefore uptake of CO_2 and nutrient.

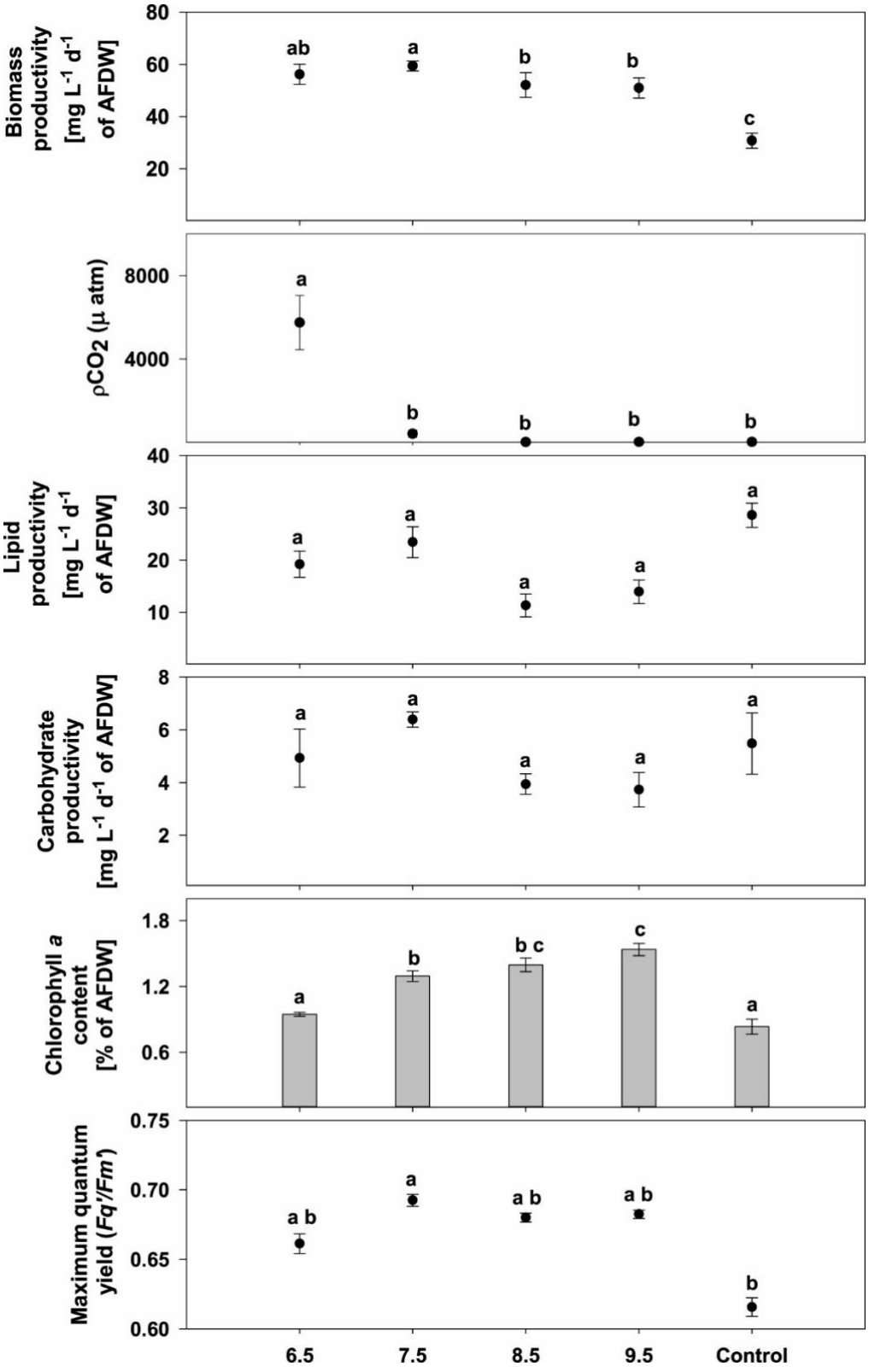

Figure 13: The effect of pH set point on the biomass productivity, pCO_2, lipid and carbohydrate productivity, chlorophyll a content and light adapted maximum quantum yield (Fq'/Fm') of T. suecica without CH_4 in the test gas. Data presented are mean values ± S.E. (n = 4). The same letter above each point and column indicate no significant difference (One Way ANOVA, P > 0.05) (Reproduced from [70]).

4.3.2 CO_2 capture efficiency and Nutrient Uptake of *T. suecica* Cultures

Table 13 shows CO_2 capture efficiency of the algae at different pH set points. The average CO_2 capture efficiencies of *T. suecica* cultures varied between 83% and 94%, at pH 6.5 and 7.5, respectively. This maximum efficiency at pH 7.5 corresponded to the highest biomass yield. However, no significant differences were observed in CO_2 capture efficiencies between pH 7.5 and 8.5 (One Way ANOVA, $P>0.05$), and similarly between pH 6.5 and 9.5. The biomass concentration of *T. suecica* cultures with CO_2 addition was significantly higher (One Way ANOVA, $P < 0.05$) than the control, in which CO_2 was substituted with atmospheric air sparged via an air pump. The average organic carbon content of dry biomass was found to be 40.7 ± 1.96% at the different pH set points. The organic carbon fixed in biomass ranged between 20.7 and 24.2 $mgC.L^{-1}.d^{-1}$ at pH 9.5 and 7.5, respectively and were not significantly different at the different pH set point values (One Way ANOVA, $P>0.05$, Table 13).

Nitrogen and phosphorus consumption rates during the algae growth experiments are summarised in Table 13. Nitrogen concentrations decreased by 92 - 99% at the end of the experiment period for all conditions. The highest nitrogen uptake rate was at the pH 8.5 set point and was 50% higher than at the pH 6.5 and 7.5 set points (One Way ANOVA, $P<0.05$ and Table 13). The phosphorus uptake rate appeared to vary between the different pH set points, but not significantly (One Way ANOVA, $P>0.05$ and Table 13). Importantly, the ADPE added to supply nutrients did not show any apparent inhibitory effects on the growth of *T. suecica*, in line with the findings of Ward et al. [42].

The COD removal rate was also measured during cultivation to determine the concentration of oxidizable substrates present; results are summarized in Table 13. The COD removal rate appeared to increase with increasing pH, with the pH 8.5 set point showing the highest COD removal rate at 152.4 $mg.L^{-1}.d^{-1}$. However, COD removal rates were not significantly different between the pH 7.5 and pH 8.5 set points (One Way ANOVA, $P>0.05$) (Table 13).

Biogas-based microalgae cultivation systems produce oxygen as a photosynthetic and metabolic co-product that may intoxicate the culture and inhibit biomass growth and CO_2 uptake from biogas. Increasing the CO_2:O_2 ratio by adding more inorganic carbon (e.g. by adding more raw biogas) is a strategy to minimise photosynthetic inhibition by the oxygen byproduct [95]. Experiments in this project maintained high concentrations of inorganic carbon, e.g. CO_2, HCO_3^- and CO_3^{2-} (up to 200 ± 47.5 $mg.L^{-1}$)(Table 13) and therefore would have minimised the risk of oxygen intoxication. Additionally, high COD of the culture media also would have contributed to removal of dissolved oxygen by biological oxidation; thereby, further minimising the negative effects of oxygen evolution on the *T. suecica* culture.

Table 13 Average (mean ± S.E., n = 4) CO_2 capture, organic carbon content, total COD and nitrogen and phosphorus removal rates at different pH limits and without CH_4 in the test gas. The same letter in each column indicate no significant difference (One Way ANOVA, P>0.05).

	CO_2 supply	CO_2 capture [%]	Carbon fixed in biomass [mg C $L^{-1}d^{-1}$]	COD [mg L^{-1}]		TN removal [mg $L^{-1}d^{-1}$]	TP removal [mg $L^{-1}d^{-1}$]
				Day 1	Day 8		
6.5	+	83%	22.9±1.58^{a}	2173.3±212.3^{ab}	2396.7±106.8^{ab}	1.39±0.58^{c}	0.08±0.03^{a}
7.5	+	94%	24.2±0.76^{a}	2020.0±0.0^{a}	1823.3±171.7^{b}	1.51±0.79^{bc}	0.07±0.01^{a}
8.5	+	94%	21.2±1.49^{a}	3260.0±0.0^{b}	2193.3±450.6^{ab}	3.00±0.53^{d}	0.03±0.00^{a}
9.5	+	88%	20.7±1.15^{a}	2450.0±0.0^{ab}	3180.0±40.4^{a}	2.19±0.81^{a}	0.03±0.00^{a}
Negative control*	-		13.4±1.86^{b}	2206.7±150.6^{ab}	3260.0±150.4^{a}	2.41±0.85^{ab}	0.11±0.02^{a}

Carbon fixed in biomass was obtained as product of the overall organic carbon content and biomass productivity. Removal rates were calculated as the ratio of the difference between the initial and final values, and the cultivation duration. TN means total nitrogen; TP means total phosphorus. *Negative control was aerated with a constant supply of air and did not have a controlled pH.

4.3.3 The effect of CH_4 on *T. suecica* Growth Performance

Table 14 shows biomass productivity and *Fq'/Fm'* values for experiments using the CH_4/CO_2 mixture compared to experiments using the N_2/CO_2 mixture. There was a short-term decrease in *Fq'/Fm'* values at the beginning of the CH_4/CO_2 experiments, which may have represented an acute response to new culture conditions. However, the system recovered and, across the whole experiment, CH_4 in the test gas did not show a significant effect on the growth rate or Fq'/Fm' values of *T. suecica* at the pH 7.5 set point (t-test, $P > 0.05$).

Table 14 Biomass productivity and Fq'/Fm' (filled circle) of *T. suecica* culture aerated with 80% CH_4/20% CO_2 and 60% N_2/40% CO_2 for a cultivation period of 5 days. Cultures were set at pH = 7.5 using a pH stat system (data are average ± SE, n = 4) [70].

Gas Composition	Biomass productivity [mg $L^{-1}d^{-1}$]	Effective quantum yield [Fq'/Fm']
80% CH_4, 20% CO_2	61±4	0.697
60% N_2, 40% CO_2	52±1.5	0.711±0.008

4.4 Application of Research

4.4.1 On farm Integration

The result of the present study clearly indicates that the microalga *T. suecica* can grow efficiently on 40% CO_2 from biogas mixtures and is not inhibited by CH_4. The results also show that *T. suecica* utilised nutrients available in piggery effluent for growth.

The ability of microalgae (e.g. *T. suecica*) to consume nutrients from digestate whilst sequestering CO_2 from biogas means that systems can be designed for efficient microalgal production. Specifically, microalgal plants can be co-located with biogas facilities to utilize the CO_2 in biogas as a source of carbon for effective biomass production, resulting in the reduction of raw material costs associated with carbon capture, storage and transportation, and unlike other carbon capture measures such as absorption by organic solvents [34, 97]. An example flow sheet showing the integration of micro-algal biogas treatment systems on-farm is shown in Figure 14.

Algae are considered a value-added by-product of the process, with potential applications as an organic fertilizer, a commercial aquaculture feed or as feed for livestock. However, the feasibility of algae processes is heavily influenced by the market value of the algae product. The integrated biogas-effluent treatment process developed in this project did not focus on maximising the market value of the algal product, but rather on reducing the costs of production. The cultivation

costs for microalgae can vary more than an order of magnitude. The integrated biogas-effluent algae treatment process does not require chemical fertilizers or CO_2 costs, which can make up approximately 76.2% of the total raw material cost for producing microalgal biomass [98]; therefore, the biogas-effluent algae treatment has the potential to greatly reduce the production cost of algal biomass.

In addition, a biogas-based microalgae cultivation system removed >90% of CO_2 from the biogas. When applied to piggery biogas with a CH_4 content of 60-70% and a CO_2 content of 30-40%, the treated biogas would have a CH_4 content of 94 to 98%. If piggery biogas was upgraded using algae the resulting biomethane is expected to meet concentration thresholds for use as a transport fuel (after compression) or to export biogas into centralised natural gas grids [28]. This has the potential to increase the usage options and the potential value of piggery biogas.

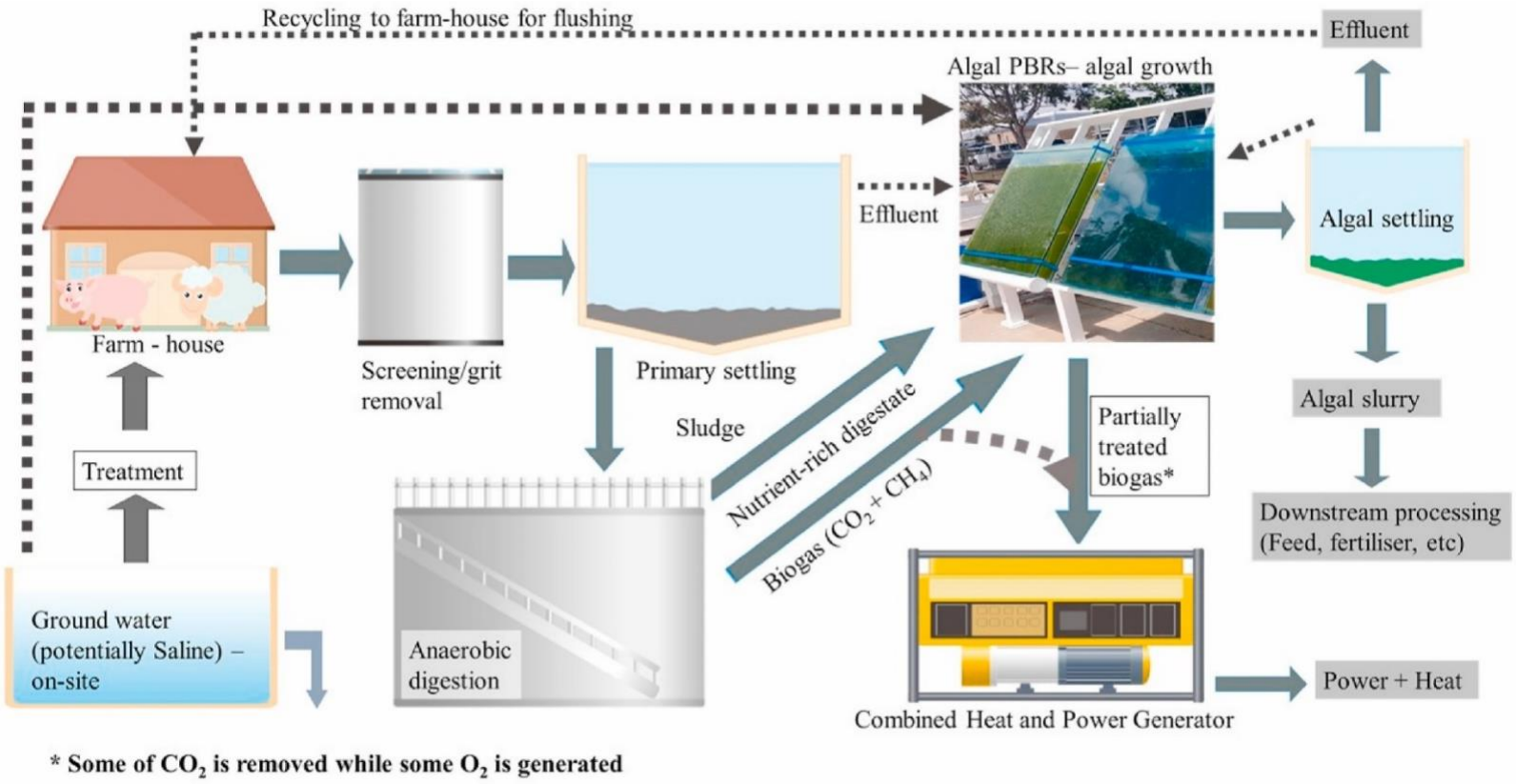

Figure 14 Example flow sheet integrating micro-algal biogas treatment system on-farm (Reproduced from [70]).

4.4.2 Scale-up Example

Table 15 shows the estimated sizing and productivity of an on-farm integrated biogas-piggery effluent treatment process using *T. suecica*. The case study was based on average daily gas production for an Australian piggery estimated at 0.08 $m^3.SPU^{-1}.d^{-1}$. For a 500 SPU piggery the summer biogas production of 82 $m^3 \cdot d^{-1}$ (for 50 $m^3.SPU^{-1}.yr^{-1}$) was used (as described in Section 2.2). For a biogas containing 30% CO_2, this corresponds to a daily carbon dioxide load of 44.4 kg (as CO_2) and a daily carbon load of approximately 12kg.d^{-1}.

Based on the results in this project, the approximate size of a high-rate algal pond would be 500 m^3, occupying a footprint of 1,700 m^2. The high-rate algal could produce an estimated 11 tonnes of biomass from a 500 SPU piggery per year from treatment of the biogas. Nutrient uptake results suggest only a small portion of the effluent nutrients at the piggery (~6% nitrogen and ~1% phosphorous), would be removed during the process, partly due to the very high nutrient content of piggery effluent (i.e., the high N to VS ratios) and the high lipid content/relatively low nutrient content of the algae product. Therefore, the integrated biogas-effluent algae process is not a complete treatment solution.

Table 15 Estimated sizing and productivity of an on farm integrated biogas, effluent treatment process using *T. suecica*.

Algae Performance	**Unit**	**Algae Process**
Biogas Productivity	$mg.L^{-1}.d^{-1}$	60
Carbon fixation rate	$mgC.L^{-1}.d^{-1}$	24
Nitrogen uptake rate	$mgN.L^{-1}.d^{-1}$	1.5
Phosphorus uptake rate	$mgP.L^{-1}.d^{-1}$	0.07
Raceway depth	m	0.3
500 SPU Case Study Inputs		
Annual biogas flow	$m^3.SPU^{-1}.y^{-1}$	50
Annual biogas flow	$m^3.y^{-1}$	25,000
Daily biogas flow (summer)	$m^3.d^{-1}$	82
Biogas CO_2 content	% CO_2	30
Daily CO_2 flow (summer)	$m^3.d^{-1}$	24.7
Daily carbon dioxide load	$kg.d^{-1}$	44.4
Daily carbon load as CO_2	$kg.d^{-1}$	12.1
Effluent Nitrogen load	$kg.SPU^{-1}.y^{-1}$	10.5
Effluent Nitrogen load	$kg.y^{-1}$	5,260
Effluent Phosphorus load	$kg.SPU^{-1}.y^{-1}$	2.5
Effluent Phosphorus load	$kg.y^{-1}$	1,275
500 SPU Case Study Sizing and Performance		
Volume of Algae Pond required	m^3	500
Surface Area of Algae Pond	m^2	~1700
Biomass produced	$kg.yr^{-1}$	~11,000
Nitrogen removed	$kg.yr^{-1}$	~300
Phosphorous removed	$kg.yr^{-1}$	~13

5 Limitations/Risks

Notes on the development of PPB desulphurisation technology:

- There are no specific limitations on the Australian region where the technology could be applied; however, the local environmental conditions will affect the growth and productivity.
- The process achieved an average H_2S removal of 69-77% in the continuous process, for a biogas stream containing 2000 ppm (volume) H_2S, which results in a product gas with approximately 700 ppm (volume) H_2S from a single stage PPB process. Safe exposure limits for H_2S are 10 ppm (volume), therefore the treated gas still represents a health hazard.
- PPB technology developed for desulphurization is designed and operated differently to PPB technology developed to generate high-value microbial protein from a range of waste streams; the designs and economics of these PPB processes do not overlap. Conclusions in this report do not impact the development or viability of microbial protein processes in general.
- Further development of PPB-based desulphurisation is not recommended, unless irradiation costs are eliminated and compressor costs can be reduced, or the economics can be improved by utilising excess biogas-derived electricity to offset the high energy costs.

Notes on the development of algae carbon removal technology:

- This project represents early development of the biogas-effluent treatment process using batch testing. Further work is required to develop the concept into a continuous process and to validate the product biogas compositions.
- From a feasibility perspective, algae are considered as a value-added by-product of the process, with potential applications as an organic fertilizer, a commercial aquaculture feed or as feed for livestock. The specific end-use and the market value of the *T. suecica* product were not evaluated in the current project.
- Piggery biogas contains 700 to 4000 ppm (volume) H_2S. The H_2S is known to inhibit microalgal growth at high concentrations [34]. The impact of high H_2S concentrations on the algae process have not been tested in this project due to the expectation that H_2S would be removed prior to the algal treatment step.
- The piggery effluent used as a source of nutrient in the experiments was added to seawater at a ratio 20 mL ADPE per L of media. This represents a dilution factor of approximately 50. The impact of high ADPE concentrations on the algae process have not been tested in this project.
- There are no specific limitations on the Australian region where the technology could be applied; however, the local environmental conditions will affect the growth and productivity [99].
- The process can be applied to other salt-tolerant microalgal species; however, a wider spectrum of microalgae would need to be tested considering their physiological responses are species-specific [39, 40].

6 Summary and Recommendations

6.1 Desulphurization using PPB

The project was successful at proof-of-concept using PPB to treat a gas mixture containing 2000 ppm (volume) H_2S, 30% CO_2 and ~70% CH_4 in a continuous process. The process achieved an average H_2S removal of 69-77% in the continuous process, with a maximum removal of 90%. The removal efficiencies achieved in the project reduced H_2S to levels suitable for on farm uses such as boilers and CHP, and therefore represent an alternative to conventional iron oxide media scrubbers for on farm use, and does not introduce nitrogen as a recalcitrant impurity like micro-aeration biological scrubbing. However, multi-stage reactors or a secondary treatment step would be required to achieve complete H_2S removal for transport fuel uses and/or export into natural gas grids.

The PPB technology reported in this project was a proof-of-concept, not an optimised process. Initial process designs indicated a H_2S loading rate of 10 $g.h^{-1}.m^{-3}$ can be achieved, corresponding to a reactor size of less than $1m^3$ for a 500 SPU piggery. The process can run chemical-free and can integrate with existing anaerobic pond technologies. However, the cost of the PPB process was estimated at approximately \$85 kgS^{-1}, which is likely to be prohibitively high. The major cost for operation of the PPB process is the electricity used to irradiate the reactor overnight; these irradiation costs would need to be eliminated for PPB desulphurization to be viable. Research could explore the continuous process dynamics under light-dark-cycling conditions, where light is only available for ~12 hours of the day (i.e., during daylight hours), or excess electricity from a biogas generator could be considered to offset energy costs.

PPB biomass was generated in the process. While protein rich biomass is a potential high-value by-product of PPB technology, the production rates within the process were very low and not sufficient to generate any significant revenue to offset the high treatment cost.

6.2 Carbon Dioxide Removal using Microalgae

The project was successful at proof-of-concept using the marine algae *T. suecica* to remove CO_2 and effluent nutrients in a batch biogas-effluent treatment process. The process achieved a CO_2 removal up to 94%. When applied to piggery biogas, the treated biogas could have a CH_4 content of 94 to 98%, making the upgraded gas suitable for use as a transport fuel (after compression) or to export biogas into centralised natural gas grids. Nutrients were removed from the effluent during treatment. However, due to the high nutrient content of piggery effluent, only a small portion of the available nutrients (~6% nitrogen and ~1% phosphorous) appeared to be required to support sequestration of all CO_2 in piggery biogas.

The process supported relatively good biomass productivity (59.8 $mg.L^{-1}.d^{-1}$). Most importantly, no inhibitory effects were seen from the CH_4 content of the synthetic biogas. The next stage of development could be to explore a continuous process, possibly at pilot stage, to clarify the biomass yields, carbon uptakes rates, and

harvesting costs, to allow a more detailed assessment of the viability of biogas-based microalgae cultivation systems.

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