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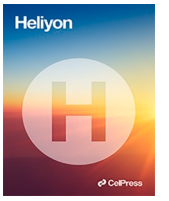

Research article

Investigations into valorisation of trade wastewater for biomethane production

Jake A.K. Elliott *, Andrew S. Ball, Kalpit Shah

ARC Training Centre for the Transformation of Australia's Biosolids Resource, RMIT University,Bundoora 3083, Australia

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ABSTRACT

Biogas production from wastewater is one way that industrial sites can work towards the UN Sustainable Development Goals, while recovering a valuable resource. The objective of this study was to investigate the suitability of data collected by municipal wastewater service providers as a method of classifying and screening waste producers as potential sites for biogas resource recovery by anaerobic digestion. Industrial wastewater samples, including raw effluent and treated waste ready for discharge, were examined, and biomethane potential assays performed. Results of chemical analysis and lab-scale digestion were compared to historical service provider data, and patterns were observed. Biomethane yields of up to 357 mL/gVS and 287mL/gVS were achieved from raw and treated effluent respectively. Digestion at the top four prospects could produce over 4690 GJ of methane and save $47,000 in natural gas costs, offsetting 490 tonnes of CO_2 equivalent annually. These streams, from logistics, waste management, food and animal product businesses, combined high levels of degradable substrates and low levels of inhibitory components. While it is unlikely that this type of screening program can be completely accurate, certain parameters, including high sodium concentration, are applicable for discounting the potential for biogas production. This knowledge can be a valuable tool in the process of selecting sites for future resource recovery, therefore increasing the uptake of these processes, resulting in economic, environmental, and climate change mitigation benefits.

1. Introduction

Liquid industrial waste, if not treated correctly, can have several negative impacts on health, infrastructure, and the environment, including eutrophication of waterways, destructive deposits in pipes, and the spread of disease [1–3]. Municipal wastewater treatment plants are unable to treat all liquid industrial wastes. Because of this, water authorities require that trade waste meets certain standards before release to sewer or the environment, and often implement programs to monitor compliance with these requirements, through analysis of the characteristics of trade waste produced by businesses within their catchment [4]. These standards may include pH, and the amount and concentration of biochemical oxygen demand (BOD); solids; fats, oils and grease (FOG); metals; and other inhibitory substances [5]. To ensure that their trade waste is accepted by the municipal water authorities, or to limit higher costs associated with disposing of inhibitory substances, businesses may elect to implement treatment solutions. Depending on the properties of the raw effluent, different treatment methods are utilised at industrial sites. Screens, strainers, and settling tanks can separate particles and oils based on physical properties at low expense [6]while chemical dosing can neutralise pH. At a higher operating and capital cost,

* Corresponding author.
E-mail address: s3511122@student.rmit.edu.au (J.A.K. Elliott).

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Table 1
Wastewater sample summary and Initial sample physico-chemical analyses. Numeral in sample number denotes site, letter denotes treatment.[a] TDS not measured for this sample.[B] COD exceeded this value, limited by measurement technique.

Sample Number	Industry	Quality	Annual discharge volume (ML)	COD (mg/L)	VS (mg/L)	TDS (mg/L)	pH	PO4 (mg/L)	TotN (mg/L)	TKN (mg/L)	Na (mg/L)	Inoculum per reactor (mL)	Substrate per reactor (mL)
0I	WWTP	Inoculum sludge		32,200	21,383	[a]	7.49	1540	1590	1568	4	77	**123 (milliQ water only)**
1T	Edible fats	Treated	196	1086	614	3140	6.331	170	8	0	2.1	44	**406**
1U		Untreated		44,900	7430	4255	6.02	272	11	5.3	2.1	254	**196**
2T	Brewery	Treated	596	5530	1864	1896	4.021	63	49	41.6	0.9	111	**339**
2U		Untreated		13,800	3778	3254	3.887	3.47	93	90.03	0.9	179	**271**
3T	Logistics	Treated	24	2880	874	2426	6.718	53	65	57.4	1.7	60	**390**
3U		Untreated		9710	5556	5174	12.29	72	83	73.2	1.5	222	**228**
4U	Chemical manufacturing	Treated	150	20	84	213	6.887	10	4	0	0.2	7	**443**
5T	Food	Treated	12	9210	3332	2883	6.324	170	103	93.5	1.5	166	**284**
5U	manufacturing	Untreated		22,200	6982	2986	5.386	263	239	227	1.8	247	**203**
6T	Chemical manufacturing	Treated	30	1071	384	419	6.427	34	18	10	0.3	28	**422**
7T	Animal products	Treated	3	>100000[b]	19,460	331,900	6.773	3.06	870	859.7	310	271	**79**
8T	Chemical manufacturing	Treated	2	10,000	1326	1757	6.496	10	0	0	0.4	85	**365**
9T	Waste	Treated	17	3650	1173	2345	5.627	2.46	50	48.56	1.2	77	**373**
9U	management	Untreated		17,700	9992	8636	12.16	3.34	19	12.8	2.4	286	**164**
10T	Chemical manufacturing	Treated	6	2560	615	1181	6.214	235	8	6.91	0.6	44	**406**
11T	Chemical manufacturing	Treated	635	140	443	1249	7.908	1.36	9	0	0.6	32	**418**
12T	Animal products	Treated	141	2000	211	681	6.959	16	96	95.26	1	16	**434**
12U		Untreated		2920	1109	1802	6.715	95	254	251.97	1.4	73	**377**

aeration can degrade some chemicals, and dissolved air flotation (DAF) can sequester suspended solids into sludge [7], for disposal in landfill.

These approaches tend to see wastewater as an additional cost, and a problem to be overcome. However, it is becoming more common to see these streams as a resource to be utilised [8]. Nutrients [7], recycled water [9], and energy [10] are potentially valuable products when waste treatment is approached with a value mindset. One of the most studied and widely applicable [11] methods of energy recovery from trade wastes is the formation of biogas through anaerobic digestion. In practice, there are many different variants of this method, including using different operating temperatures [12,13], different feedstocks and co-digestion [14,15], and separation techniques [12,16,17]. Despite operational differences, these methods share a common underlying process, where a methanogenic microbial community converts carbon in the waste stream into methane under anaerobic conditions [18]. This biogas can be used directly as a feedstock for industrial boilers [19], or can be scrubbed to remove hydrogen sulphide and other impurities [20]and utilised for electricity generation or dispatched to the natural gas network. Producing and utilising biogas from wastewater streams helps work towards the UN Sustainable Development Goals (SDG) of clean water and sanitation (SDG 6), affordable and clean energy (SDG 7), sustainable production (SDG12), and climate action (SDG13).

Currently, this potential resource goes largely unharnessed at an individual business level; instead the effluent, weakened by pre-treatment on-site, and diluted by other sewage, may undergo digestion at a municipal waste-water treatment plant (WWTP) to remove remaining BOD and to produce gas [21]. Although the full economic and environmental impacts are currently unknown for industrial wastewater, biogas production from the combined, dilute waste stream is generally not as efficient as that of raw effluent when considering the pre-treatment, as well as reactor volumes and retention times required [22]; in addition, greenhouse gas emissions of water treatment facilities in Victoria reach almost one million tonne CO_2 equivalent per annum [23].

In contrast, anaerobic digestion on suitable sites could present advantages to waste producers and municipal water authorities. On the one hand, the producer may be able to reduce ongoing waste fees and energy costs, and lower their greenhouse gas emissions, while the municipal water authority could benefit from a lower volume of water requiring treatment at the WWTP. The cost of repairing sewer corrosion is estimated to reach hundreds of millions of dollars in Australia alone [24]. Reducing transport costs and damage to utilities are possible positive outcomes of diverting strong wastewater at the source. Unfortunately, at present, individual premises rarely have the technical capability or capital investment required for on-site resource recovery.

Given these potential mutual advantages, municipal water authorities may be incentivised to partner with individual customers to capture biogas on-site. However, with the sheer number of businesses which may exist within a catchment, it may not be practical to approach each individually. As such, a screening and classification system would be a useful tool in identifying those waste producers with the most potential for biogas production, especially if a producer already stores historical chemical and physical data. Some studies have mapped potential areas for large scale co-digestion power plants using high level theoretical data based on industry type [10], while others have devised systems of classification for wastewater to visualise recycling and industrial symbiosis opportunities [25,26] However, the practical screening of raw and pre-treated wastewaters to determine the suitability of individual small-scale biogas fermentation has, to the authors' best knowledge, not been reported in the literature. Recent studies have investigated and compared digestion of treatment plant sludges [27], but not substrates from a step earlier in wastewater treatment, i.e. at the site of wastewater generation.

To fill this knowledge gap, this study performs biomethane potential tests on a range of industrial wastewater substrates. Using these experimental results, and the related historical water authority data, we aim to create a resource recovery classification scheme, and elucidate some of the issues with this as a method for finding potential sites of interest for biogas fermentation, identifying areas where improvements need to be made for accurate screening to be possible. The purpose of an accurate screening model is to promote the uptake of anaerobic digestion technologies on industrial sites, minimising greenhouse gas emissions and utility costs, while capturing value from a previously untapped source. Limitations here include the lack of complete sets of municipal water data; also, true impacts of full-scale digestion may not map perfectly to the classification scheme. Investigations into a classification scheme for municipal wastewater treatment corporations to identify targets for resource recovery are seldom found in literature, and the use of real samples from a range of sites underpins the novelty of this study.

2. Materials and methods

2.1. Sample selection and collection

To test a wide range of substrates, access to routine trade waste analysis data was granted by Greater Western Water (GWW), a municipal water authority servicing a large part of Melbourne, Australia. This de-identified data, broken down into industry groups such as food manufacturing, waste management, and chemical production, included various composite and single point measurements such as total dissolved solids (TDS), total suspended solids (TSS), pH, BOD, nitrogen, and metals. Sites of interest were manually selected to achieve a wide range of industry groups and chemical profiles, within the constraints of the ability to collect physical samples (Table 1). As water service provider information relates to wastewater as it enters the sewerage system, substrate was taken from each business at the last point before discharge, after all on-site treatments were carried out. When possible, samples were also taken at the point before treatment, to allow for comparison and investigation into substituting treatment processes. To maintain confidentiality of trade waste customers, samples were taken by GWW technicians during scheduled visits. Samples were stored at 4 °C in appropriate vessels, before being transferred to the study laboratory for testing and digestion as described below. Upon receipt, one vessel of each sample was frozen at −18 °C for microbial analysis. Mature waste activated sludge (WAS) from an operational water treatment plant was supplied from the Koo Wee Rup Municipal Wastewater Treatment Plant, operated by South East Water

2.2. Chemical and physical analysis

Both the sludge and the supplied wastewater were analysed for chemical and physical properties (Table 1). Standard methods were used to calculate TDS, TSS, and VS [28]. pH was measured using a Hanna Instruments HI5221 pH meter. COD, phosphorus, total nitrogen, nitrates, nitrites, and ammonia were measured using spectrophotometric methods. COD analysis was performed following the USEPA Reactor Digestion Method, as Method 8000 from Hach [29], using COD (HR) reagent vials. Phosphorus was analysed using Hach's Method 8190, USEPA PhosVer® 3 with Acid Persulfate Digestion Method [30], using the appropriate Test 'N Tube™ Vials. Total nitrogen, nitrate, nitrite, and ammonia were measured using Persulfate Digestion Method (Method 10,071) [31], Chromotropic Acid Method (Method 10,020) [32], Diazotization Method (Method 10,019) [33], and Salicylate Method (Method 10,031) [34] respectively, and the applicable Test 'N Tube regent kits. Each test utilised the Hach DR900 Colorimeter and DRB200 Reactor heating block as necessary. Salinity was measured using a Horiba Scientific LAQUAtwin-Salt-11 salt meter. When required due to chemical concentration exceeding measurable ranges, samples were diluted with milliQ type 1 water.

2.3. Anaerobic digestion

To measure the biological methane potential (BMP), anaerobic digestion of each feedstock was performed in parallel. Sludge and wastewater samples were individually homogenised, before aliquots of sludge inoculum and substrate were added to each digester, resulting in a specified inoculum to sludge ratio (ISR) (Table 1). ISR can be a major factor in the levels of methane produced in a BMP test, especially due to the buffering effect that ISR can have against acid inhibition. While effective ISRs can range, depending on the solids concentration of the reactor vessel, and whether the substrate itself has a buffering effect [35]. Although there are numerous studies determining the optimal ISR for particular inoculum/substrate pairs, it would not be sensible in this study to perform the multiple rounds of digestion required to find this, particularly as the aim is a screening comparison between substrates. The final ISR chosen was 3.5 on a volatile solids weight/weight basis. Inoculum to sludge ratios in this range have been shown to allow for maximum biogas production in batch tests on a range of feedstock, using WAS as inoculum [15,36], providing some buffering from inhibition by low pH. A total liquid volume of 450 mL was used for all substrates tested except sample 7T, which was limited to 350 mL due to high VS concentrations. Although constant inoculum volume is usually employed in BMP tests [37], constant total volume was instead used in this study to allow for consistent reaction vessel size, and therefore consistent mixing and thermal properties, despite the large range in substrate VS content. No dilution of substrate was undertaken, to reflect that these are real waste streams, and would ideally be fed to reactors on site without dilution, to minimise reactor footprint. Variability in gas production due to different initial inoculum quantity is an important factor when considering practical application of on-site digestion. A control mixture of the inoculum was also developed. This digester was primed with 77 mL of inoculum and 123 mL milliQ water, to produce a workable viscosity. Each sample/inoculum mixture, including the control, were added in duplicate to a 500 mL serum bottle, which was then sealed with a rubber stopper, and connected via vinyl tubing to a water displacement gas capture rig.

Every digester bottle was maintained at 38 °C using two large water baths and Thermoline Unistat 11 Immersion heater circulators. The gas capture unit was housed in a climate-controlled laboratory at 24 °C. During the study, every sample was routinely mixed by swirling the individual bottles. Once the gas captured was of an adequate volume for analysis, it was extracted with a 60 mL syringe, and measured using a Geotechnical Instruments GTIBM2K-E000 Biogas Check Analyser. CH_4, CO_2, and O_2 percentages were recorded alongside total volume. Digestion continued for a period of 77 days. Samples 1T, 4U, 6T and 8T were randomly selected to be digested in triplicate, while the remainder were digested in duplicate. The overall experimental run was considered complete when daily methane production of every sample had decreased to less than 1% of the cumulative total for a period of at least one week.

2.4. Post-digestion analysis

After the digestion period concluded, pH, salinity, COD, Tot-N, NO_2, NO_3, NH_3, and PO_4 were all measured using previously described methods.

2.5. Microbial analysis

Following digestion, 8 samples and the inoculum control were selected for microbial analysis. The samples were chosen to compare a selection of high yield streams (3U, 5U, 5T, 9U, 12U), low yield treated streams from the same sites (3T, 12T) and one sample that fell well outside of the trend of increased VS resulting in increased gas production (7T). Aliquots of pre- and post-digestion samples were centrifuged at 9000 RPM for 10 min to collect enough solid material. DNA was then extracted from the pellets using Qiagen DNeasy PowerSoil kits according to the manufacturer's protocol, and stored at −20 °C. To find the total bacterial copy number of each sample, qPCR was performed [38]. A master mix was made for each test of 8.2 μL PCR grade water, 10 μL 2x SensiFAST™ SYBR® No-ROX Mix (Meridian Bioscience), and 0.4 μL each of the universal 16S rDNA primers 341F (5′ CCTACGGGAGGCAGCAG 3′) and 518R (5′ ATTACCGCGG CTGCTGG 3′) (10 pmol/μL) [39], allowing for 1 μL of sample. Amplicon standard solutions (5×10^{-1} to 5×10^{-8} ng/μL) were added to calculate a standard curve through linear regression of cycle threshold values, and negative and no-template-controls were created using PCR grade water and master mix respectively. Extracted DNA solution from experimental samples were tested in triplicate. After an initial 5-min, 95 °C denaturation step, samples were subject to 40 cycles of 95 °C denaturation (10s), 55 °C annealing (30s), 72 °C extension (30s), and 80 °C primer dimer removal and signal reading (10s) using a Qiagen Rotor-Gene Q real-time PCR cycler. After the qPCR run was complete, Biorad software was used to calculate copy number per sample.

Individual measurements with values matching negative controls were removed manually.

2.6. Statistics and similarity analyses

Statistical significance of difference between the methane yields of each sample was determined using one-way ANOVA and the Tukey-Kramer post-hoc test.

Hierarchical clustering analysis is a method of visualising similarity of samples into a dendrogram. Hierarchical clustering analysis was performed using "factoextra" and "cluster" for R, and visualised using ggdendro [40–42].

Principal component analysis (PCA) is a method of multivariate analysis that has been used to identify groups of samples in many wastewater, resource recovery, and microbiology applications [43–45]. This method reduces many variables into a more interpretable form [46]. PCA was completed using R Stats [47] and GGbiplot [48,49].

Statistical significance of differences between groups was found using ANOSIM Bray dissimilarity method, and Mantel Spearman method in the "vegan" R package [50].

2.7. Benefits calculations

$$E_{CH_4\ ann} = \frac{V_{eff}}{V_{exp}} \times \frac{V_{CH_4\ exp}}{ISR + 1} \times U \tag{1}$$

The energy savings available to a site by fully utilising the biogas potential was calculated according to Equation (1), where $E_{CH_4\ ann}$ is the calculated methane production, V_{eff} is the annual volume of wastewater produced, V_{exp} is the volume of wastewater used in the experimental digester, $V_{CH_4\ exp}$ is the volume of methane produced by the experimental digester, ISR is the inoculum to sludge ratio, and U is the energy density of methane.

GHG emission diversion was calculated using Equation (2).

$$V_{rem} = \Delta c_{COD} \times \left(\frac{m_{COD\ sub}}{m_{COD\ sub} + m_{COD\ inoc}} \right) \times V_{eff} \times F \tag{2}$$

Where V_{rem} is annual volume carbon dioxide equivalent (20 year basis) diverted from the atmosphere, Δc_{COD} is experimental decrease in COD concentration, $m_{COD\ sub}$ is mass of COD in experimental reaction from substrate, $m_{COD\ inoc}$ is mass of COD in experimental reaction from inoculum, V_{eff} is annual volume of wastewater produced, and F is CO_2 equivalent production factor, using values for greenhouse gas production per unit COD from Ref. [51], and CO_2 equivalent values from Ref. [52].

3. Results and discussion

3.1. Chemical analysis

Table 1 shows that large disparities between compositions of treated and untreated wastewater from identical sites are evident. Comparison of different sites also show that treatment does not cause consistent changes between all sites. For example, sites 9 and 12 both utilise settling, cooling, oil interception, and pH neutralisation processes. At site 9, untreated waste contains 17,700 mg/L COD, which treatment lowers to 3650 mg/L. Site 12 has an initial level of 2920 mg/L COD, which is only reduced to 2000 mg/L. Raw effluent at site 9 is strongly alkaline, but treatment results in a weak acid outfall. Site 12, on the other hand, produces waste with a neutral pH,

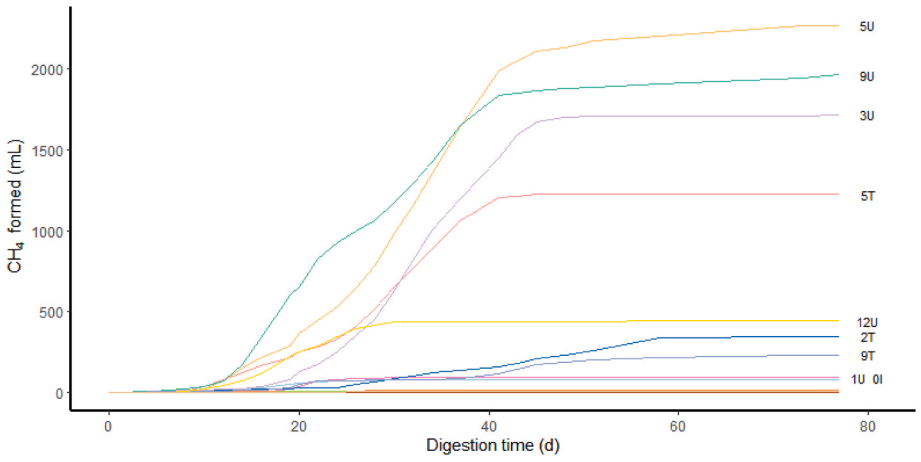

Fig. 1. Cumulative methane production over time, all samples. Samples producing <15 mL not labeled.

both before and after treatment.

3.2. Biogas production

Fig. 1 shows the absolute methane production profiles of all samples. Three untreated samples have produced the highest volume of methane per digester in the study (Table 2). The high total gas volumes produced by samples 3U, 5T, 5U, and 9U are a function of these samples' high methane yields and high VS contents.

Fitting a modified Gompertz equation to methane production [15,53–55] using non-linear least squares regression gave varying levels of fit. There is a lack of suitable generalised mathematical model for the kinetics of this process [55]. Upon visual inspection, samples 5T, 5U, 9T and 9U were notable as they did not conform to the model, despite low residual sum of squares (RSS) values. The gas formation profile of these three samples, and in particular sample 9U, resemble diauxic growth, with two periods of a higher rate and a lag phase between. This lag phase is potentially caused by VS and nutrients from the inoculum being consumed before the microbial community shifts to degradation of the added substrate [56]. Studies have found similar profiles during the anaerobic digestion of food, leaves and straw [57], and fish waste [58], among others. Low-carbohydrate, high-fat substrates in general often result in diauxic growth in BMP assays, and mixing high-carbohydrate feedstock in for co-digestion can minimise the lag period, while also increasing the relatively low methane production rates of the latter substrate [14]. Carbohydrate and fat content are unsuitable for an automated classification program as they are unlikely to be stored as numerical data at a municipal water service provider, but specific knowledge about a site's operations could be utilised after screening and early BMP assays to combine waste streams for co-digestion. Research into each unique co-digestion pairing may be required.

COD concentration increased in samples 2U, 4U, and 6T over the course of the digestion. These samples generally produced very low volumes of biogas, and it is assumed that biological processes formed some COD via reduction products [59].

3.3. Site differentiation and automated screening

To find the differences between samples, and if historical data showed a distinct group of parameters that would indicate high biogas potential, hierarchical clustering analysis of available data was undertaken. Parameters included were BOD, COD, pH, TSS, TDS, BOD:COD ratio, and sodium. As can be seen in Fig. 2, high producing samples were not related by the historical data of the effluent post onsite treatment.

Principal component analysis (Fig. 3) of the measured characteristics of the treated and untreated samples also did not show defined groups relating to methane production. Using ANOSIM for two group (high/medium or low) and three group (high, medium or low), and Mantel tests (absolute methane volume per digester, methane yield per gram VS per digester) showed no significant correlation to the variables ($p = 0.5964, 0.239, 0.5243, 0.5352$ respectively). As the automated approaches of hierarchical clustering and PCA were not able to define groups in relation to BMP, an individual appraisal of each substrate in this study is required.

3.4. Individual site appraisal and identification of parameters of interest

Fig. 4 shows the average volume of methane produced, normalised to the total concentration of volatile solids in the reactor. Recalling that sample 0I is the control sludge inoculum, samples 3U, 5T, 5U, 9U and 12U, and to an extent 2T and 9T, show a statistically significant greater efficiency in converting volatile solids to methane ($p = 0.05$). All other samples produce a statistically significant ($p = 0.05$) lower yield of methane per gram of volatile solids than the inoculum. Sample 7T was not included in this

Table 2
-. - Digestion analysis highlights.[a] Average value (standard deviation). .[b] Values impacted by initial sample measurement issues.

Sample	Reactor initial COD (mg/L)	Reactor initial VS (mg/L)	COD removal (%)[a]	CH_4 produced per digester (mL)[a]	Biogas yield (mL CH_4/gVS)[a]
0I	12,397	8232	44%	80.35 (5.52)	48.80 (3.35)
1T	4128.3	2645	23%	8.46 (9.79)	7.112 (8.222)
1U	37731.6	15,310	55%	92.71 (26.79)	13.46 (3.890)
2T	12108.6	6679	55%	348.03 (162.96)	1115.80 (54.223)
2U	21119.1	10,780	<0%	1.73 (1.73)	0.3571 (0.3571)
3T	6789.3	3609	41%	2.78 (2.78)	1.7114 (1.7114)
3U	20805.1	13,360	36%	1715.5 (56.89)	285.26 (9.460)
4U	520.6	415.3	<0%	0.04 (0.06)	0.214 (0.3027)
5T	17690.8	9991	55%	1226.28 (30.17)	272.76 (6.710)
5U	27688.9	14,890	46%	2269.38 (2.85)	338.7 (0.4248)
6T	3007.9	1691	<0%	0.54 (0.77)	0.7151 (1.011)
7T	>47503.4 [b]	20,950	48%	1.98 (1.51)	0.2694 (0.2063)
8T	14193.3	5115	6%	0.84 (0.58)	0.3644 (0.2502)
9T	8535.2	4631	53%	231.98 (67.75)	111.31 (32.51)
9U	26915.6	17,230	41%	1962.4 (1.98)	253.07 (0.2551)
10T	5458.1	2646	34%	12.68 (12.13)	10.649 (10.19)
11T	2419.8	1932	38%	10.98 (4.51)	12.629 (5.190)
12T	3073.8	963.8	71%	6.6 (1.1)	15.21 (2.542)
12U	7669.9	4398	58%	444.93 (6.2)	2224.82 (3.133)

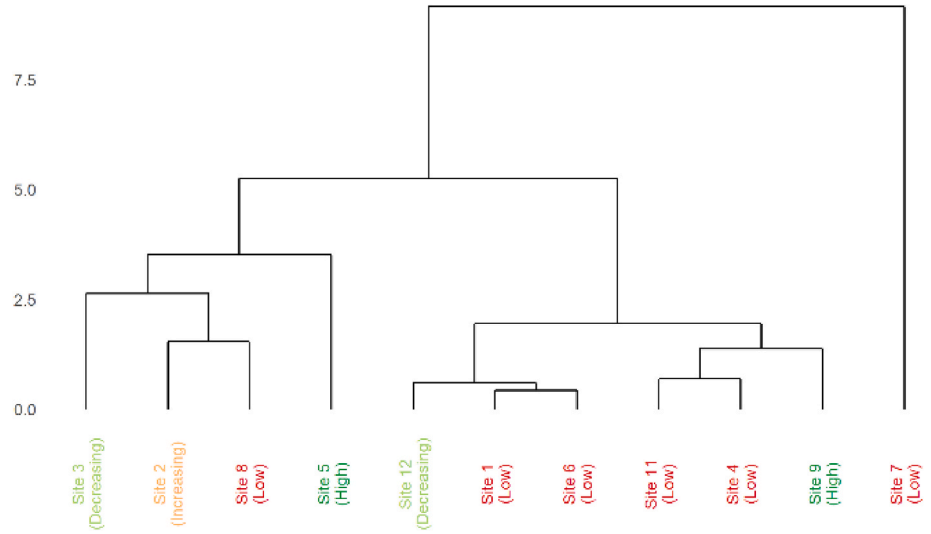

Fig. 2. Hierarchical Cluster Analysis of wastewater from the database, using Ward's minimum variance method. Gas production profiles between untreated and treated effluent are in parentheses.

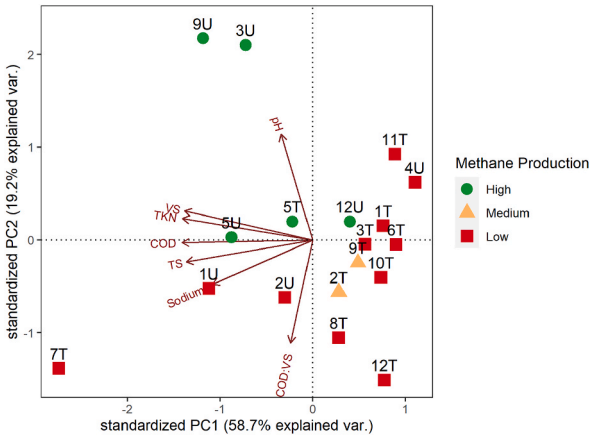

Fig. 3. PCA of experimental measurements, with numbers denoting sample codes.

statistical analysis due to the different initial volume, but the extremely low gas production can still be used as a qualitative indicator for comparison while screening.

For digestion mixes with lower levels of VS, approximately 3500 mg/L or less, it is likely that the start-up period consumed the majority of substrate before biogas could be produced [60]. Abundance of methanogens is also impacted by volatile solids concentration and organic loading rate [61]. Above 3500 mg/L, however, methane production varied almost independently of VS concentration, either as an absolute value or per unit of volatile solid. From this finding, a lower limit of 3500 mg/L VS can be implemented in a screening program. VS is likely to be measured by a water service provider but is also highly likely to be lowered in on-site pre--treatment, limiting the full applicability of this parameter.

Of the low producing outliers, sample 7T has clearly been inhibited by a high salt content, calculated at over 7% in the reaction mix. Of the other samples, all salt concentrations are within the range that may improve COD solubility, and therefore biogas yields [62]. Sample 1U, another substrate with high VS, but very low gas production, may be limited by the refractory COD content, indicated in this case by the relatively high ratio of COD to VS. Other digesters that may have suffered this type of inhibition are 7T, 8T, 10T, and 12T. As sodium is frequently measured by water service providers, but not commonly removed during pre-treatment, a high concentration of salt is a reliable indicator in a screening program to rule out certain sites as being suitable for biogas production. Refractory COD may not currently be measured by water service providers, so more research into its measurement and effects on biogas production is required.

Wastewater for sample 2U initially had the lowest pH of all substrates, a distinction that was carried through to final pH measurement of the digested samples. As methanogens are limited by low pH [63], the low levels of methane produced are not unexpected. Sample 6T also had a relatively low pH and low gas production. In contrast, samples 3U and 9U had initial alkaline conditions that

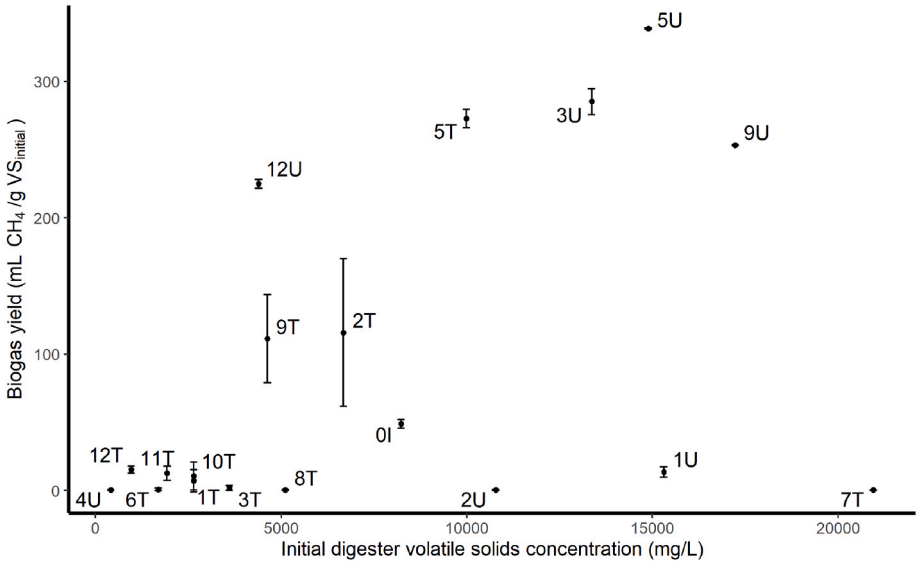

Fig. 4. Gas production and volatile solids concentrations. Error bars show standard deviation of biogas yield over replicate samples.

dropped into a range conducive to methanogens by the end of the digestion. Samples 5T, 5U, and 12U, all of which were acidic but produced high levels of gas, show that pH can impact methane production. This can be mitigated through the buffering effect of alkalinity added through substrate or inoculum [64]. pH is one of the most common parameters to be measured, so historical data is likely abundant. However, it is also frequently treated on-site, so discharge data is not indicative of raw effluent. In addition, alkalinity has a greater effect on pH control in digestion reactions than the original pH and would therefore be more useful for screening programs. Unfortunately, it is only occasionally recorded in historic data.

These results also show that biogas production is not limited to one industry type, so any investigation into resource recovery suitability should not be restricted by industry. Biomethane potential assays as performed in this study are important to fully understand the suitability of a waste stream for digestion.

3.5. *Effects of on-site pre-treatment*

Fig. 5 highlights the relationship between treated and untreated wastewater at each site. Treatments range from straining and settling through oil and grease interception and dissolved air flotation. Pre-treated wastewaters have lower COD and VS and generally

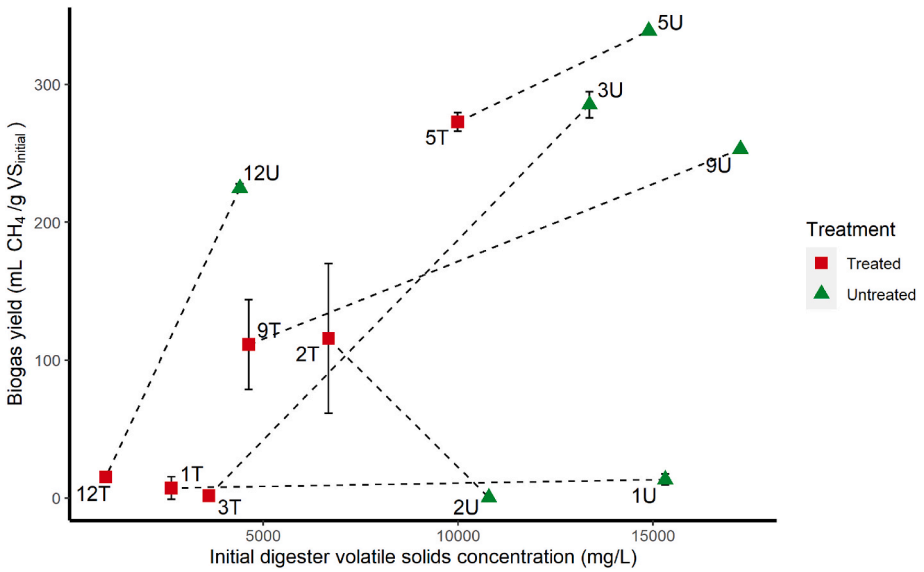

Fig. 5. Effects of on-site pre-treatment on biogas production. Error bars show standard deviation of biogas yield over replicate samples.

a pH closer to neutral than untreated wastewater from the same site. This is expected, as these parameters are some of the leading components to be corrected before release to sewer [5].

Samples 9U and 9T are the untreated and pre-treated wastewaters respectively from a waste management business, and samples 5U and 5T are untreated and pre-treated streams from a food processor. While both premises use straining, settling, and pH neutralisation for their treatment, the waste management site utilises oil interception, while the food manufacturer uses dissolved air flotation (DAF). Both show diauxic patterns. Sample 9T shows longer lag phases and later gas production peaks, as well as lower methane production in the lull between peaks compared to sample 9U. As ISR between the digestion mixtures are the same in relation to VS, this indicates that the untreated feedstock contains more readily digested solids, a similar finding to other studies involving biogas production from treated wastewater [65]. This is somewhat counter-intuitive, when oil interception should result in a relatively lower fat substrate, and single-phase digestion [14], but can be explained by the influence of the other treatment processes. The food wastewater also showed diauxic growth, but with only minimal discernible differences between peaks. Although DAF treatment removes COD, BOD, and solids in un-equal proportions [66], the digestibility of the substrate did not appear to change. In most sample pairs, however, treatment lowered biogas production. Although some literature on the anaerobic digestion of the concentrated waste stream from processes like DAF [67,68] is available, further studies comparing the viability of raw wastewater and DAF sludge as feedstock are required.

The only case in which untreated effluent formed less biogas was Site 2: a brewery. It is likely that the raw effluent contained inhibitory chemicals, e.g. ethanol [69], and that most of the biologically available carbon was spent. If so, it is possible that treatment processes undertaken onsite could have removed the inhibitory compounds [70], and converted some carbon into a biologically available form. While pre-treatments have been shown to increase biogas production [71], further study into the mechanism and reproducibility of this result is required.

3.6. Select microbial analysis

Fig. 6 shows, for a selection of samples, the 16S copy number per mL of the original wastewater, and that of the digestion mixtures after digestion. This was performed to provide a high-level overview of microbial activity. There is little correlation between BMP and copy number, whether of the sample, or the digestion mixture. These inconsistencies are likely because testing was not specific to methanogens. Measuring methanogens specifically could indicate a mechanism and relationship between microbial abundance and methane yields, and is an area for future study.

In general, copy number in the post-digestion mixture shows a correlation with initial reactor VS concentration. Because of the high ISR, the inoculum volume strongly influences the initial VS concentration, and copy.

Number. All digesters tested showed a decrease in copy number after digestion. Studies into bacterial communities in reactors over time, and particularly the relative abundance of methanogens, are widespread for various substrate and treatment combinations [72–74]. Similar investigations into the samples from this study are warranted.

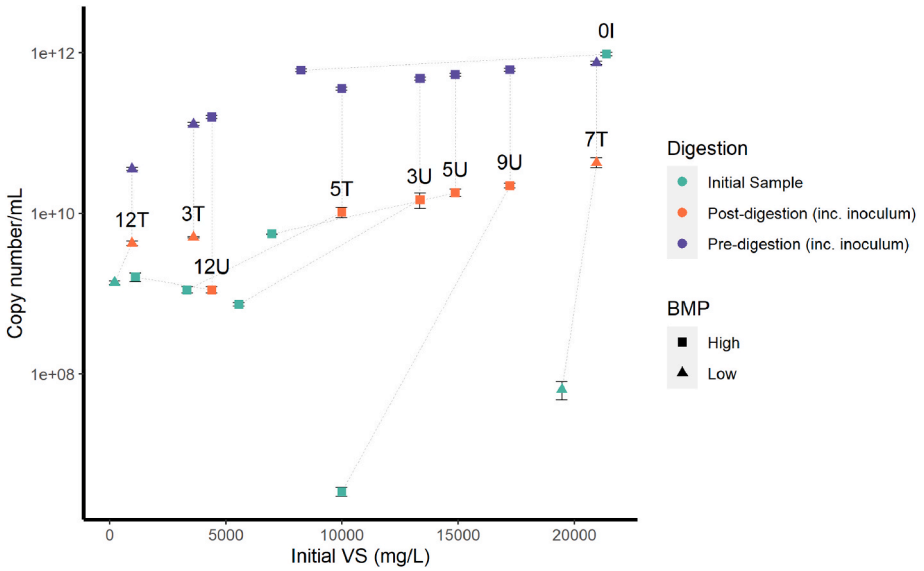

Fig. 6. 16S qPCR results. Filled points samples with high BMP, outlines only are low producing samples. Error bars denote standard error from qPCR replicates. Dashed lines connect same samples. For initial samples, VS is that of the sample. For post-digestion samples, VS is that of the initial reaction mixture of inoculum and substrate. Error bars show standard deviation of replicates.

3.7. Gas utilisation and benefits

An important factor in take-up of non-traditional treatment methods is the economic outcome for a business, i.e. the cost savings should ideally be greater than the capital and operating expenses. As biogas is suitable as a direct feedstock for industrial boilers [19], one of the most significant potential savings is the cost of natural gas for water heating and steam generation. At a low enough price, biogas substitution can reduce payback periods of new drying units in dairies [75]compared to using natural gas. Another possible saving is in the cost of traditional water treatment before disposal to sewer, as the anaerobic digestion process removes COD. Separation technologies that increase biogas production while providing an even cleaner outlet water stream are also available [8].

Table 3 shows the potential economic [76] and environmental benefits from using biogas in the place of natural gas, and preventing COD leaving site and forming greenhouse gases in uncontrolled circumstances.

Bywater and Kusch-Brandt [77] examined a potentially more varied feedstock than is available on many of the sites within this study, rejecting low-yield streams, but at a similar magnitude of waste volumes. An on-site anaerobic digester was shown to approach economic viability in a subsidy-free environment, especially when considering heat, electricity, and other benefits such as use of digestate as fertiliser. Further quantification of "public goods" benefits further increase this viability. After energy savings, ability to use digestate in land application is found to be a major factor in economic and environmental sustainability in many studies [78,79]. This may be of more limited use in the urban area described in this paper, as transport costs to land application sites will naturally be higher than in situations where farms and digesters are co-located. The reduction in waterway eutrophication [78] would also not be as pronounced in the catchment examined in this study, as the entirety of the effluent stream is already captured and processed, either at municipal wastewater treatment plants, or at sealed landfill facilities. However, improvements in sewerage infrastructure condition due to decreased microbial corrosion could provide significant savings [80], although this would be difficult to quantify.

4. Conclusion

Industrial trade wastes can be an excellent target for resource recovery, particularly that of biogas generation. Large savings in terms of gas costs and greenhouse gas emission diversion are possible. Additionally, effluent that has not yet undergone pre-treatment on-site is generally a more suitable substrate, meaning biogas digestion may decrease other treatment costs.

At this stage, it appears that using historical data from water service providers to predict individual trade waste customers' suitability for resource recovery through anaerobic fermentation of biogas is ineffective. Historical data is often incomplete, and does not accurately reflect the quality of the raw effluent available As normal pre-treatment by a trade waste customer is aimed at removing BOD and solids, and biogas fermentation converts BOD into a useful product, the two processes are at cross-purposes with each other, and fermentation is often more efficient with raw effluent. Biogas production, and the microbial communities it relies on, can be restricted by many different factors. As a result of the limitations in the available data, it is not possible to create a complete and definitive system of classification for biogas production from trade wastes. However, historical data can still point towards individual effluent streams as potential feedstock at an early stage of the discovery process. In conjunction with this, qualities such as high sodium can be used to reject unsuitable sites. Biomethane potential assays for sites of interest can be carried out as part of a more tailored search.

To capitalise on these BMP assays being a part of the knowledge base of a water authority, future studies into combining substrates from geographically proximate sources are required. The augmentation of effluent shown to follow diauxic fermentation kinetics with single-phase, easily degraded substrate is an area that requires examination, to maximise efficiency and business outcomes.

Further, based on findings regarding methane potential decreasing after treatment, future studies investigating the suitability of concentrated waste separated from wastewater, for example DAF sludge, are required. Analysis of the costs and benefits of standard treatment methods, resource recovery processes, and combinations of the two, may increase industrial resource use efficiency and profitability, and decrease environmental impacts. Future work will include analysing the microbial communities associated with each sample, in particular that of methanogens; co-digestion of samples to overcome substrate limitations; and confirming suitability of waste streams using longer-term continuous digestion.

Credit author statement

Jake Elliott – Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Andy Ball – Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. Kalpit Shah – Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 3
Potential benefits from on-site digestion.[a] Soluble product of fermentation contained greater COD than initial loading. Using gas price 2021/22 YTD, March, Victoria, Australia [76],

Site Code	On-site treatment	Methane production (GJ/y)	Gas savings ($/y)	tCO2eq diverted
1	Treated	28.02	281.6	63
	Untreated	635.7	6388.79	2993
2	Treated	2231	22421.55	1951
	Untreated	26.13	262.61	n/a
3	Treated	1.173	11.79	35
	Untreated	1238	12441.9	59
4	Untreated	0.09288	0.93	n/a
5	Treated	355.3	3570.77	57
	Untreated	919.9	9245	81
6	Treated	0.2652	2.67	n/a
7	Treated	0.5144	5.17	47
8	Treated	0.03151	0.32	1
9	Treated	72.5	728.63	38
	Untreated	1395	14019.75	63
10	Treated	1.285	12.91	7
11	Untreated	114.4	1149.72	45
12	Treated	14.7	147.74	278
	Untreated	1141	11467.05	287

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