

Finding new biotechnological solutions - Advancing microalgal wastewater treatment with beneficial bacteria.

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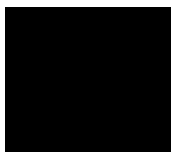
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Abstract

Microalgal wastewater treatment systems may solve several problems with traditional systems. They can cope with high nutrient loads, do not require a supplementary oxygen input, and contribute to a circular economy. However, there are still challenges presented when upscaling these systems. Certain bacterial strains can promote microalgal growth due to the mutualistic symbiotic relationships formed between these microbes. Therefore, the purpose of this study was to analyse microalgal microbiomes that have been cultivated using wastewater and identify potentially beneficial bacteria within these microbiomes with the aim to make microalgal wastewater treatment systems viable at large scales. DNA extraction, 16S rRNA amplicon sequencing, and co-cultivation experiments were conducted to analyse the biodiversity of the *Tetradismus obliquus* microbiome when cultivated on agricultural digestate, identify possible beneficial bacterial strains for use within *T. obliquus* wastewater treatment systems, and investigate the impact of specific bacterial strains on *T. obliquus* growth. The *T. obliquus* microbiomes cultivated on digestate had significantly different bacterial community compositions with lower species diversity and evenness compared to the *T. obliquus* microbiomes cultivated using a control medium. A bacterial strain belonging to the *Pseudomonadaceae* family was found in great abundance within the digestate-treated microbiomes and appeared to rapidly grow in abundance within this environment after the first day of cultivation. Co-cultivation analysis found that an isolate identified as *Psuedomonas laurentiana*, which shared significant sequence similarity to the bacterial strain belonging to the *Pseudomonadaceae* family, significantly increased *T. obliquus* growth. The isolate identified as *P. laurentiana* may therefore be suitable for use within and advance *T. obliquus* wastewater treatment systems. Further co-cultivation studies should be conducted within digestate and other wastewater environments at larger scales to determine if the consortia of *T. obliquus* and *P. laurentiana* are effective when trying to upscale *T. obliquus* wastewater treatment systems and make them more efficient.

Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

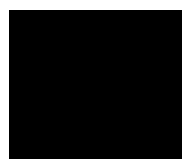


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This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

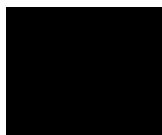


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Contents Page

Abstract	2
Declarations	3
Acknowledgments	6
List of Tables and Figures	7
Abbreviations	8
Introduction	9
Materials and Methods	12
<i>Cultivation of microalgal samples for DNA extraction</i>	12
<i>DNA extraction, and 16S rRNA gene amplicon library preparation</i>	12
<i>Bioinformatic and statistical analysis of the microalgal microbiomes</i>	13
<i>Bioinformatic analysis</i>	13
<i>Alpha and Beta diversity of the microalgal microbiomes</i>	13
<i>Abundance and functional analysis of the microalgal microbiomes</i>	14
<i>Co-cultivation of bacterial isolates and T. obliquus</i>	15
Results	16
<i>Nutrient information, collected by previous researchers, for the microalgal microbiome samples</i>	16
<i>Analysis of sequence data</i>	16
<i>Bacterial alpha diversity of the microalgal microbiomes</i>	16
<i>Bacterial beta diversity of the microalgal microbiomes</i>	17
<i>Abundance of bacterial strains within the microalgal microbiomes</i>	18
<i>Functionality analysis of the bacterial communities within the microalgal microbiomes</i>	22
<i>Co-cultivation of 8 different bacterial strains with T. obliquus</i>	25
Discussion	27
<i>Bacterial diversity within the microalgal microbiomes</i>	28
<i>Bacterial community structural diversity between the microalgal microbiomes</i>	29
<i>Abundance of taxonomic classes and bacterial strains within the microalgal microbiomes</i>	30
<i>Functionality of the bacterial communities within the microalgal microbiomes</i>	31
<i>Co-cultivation of T. obliquus and bacterial isolates</i>	32
<i>Limitations and further work</i>	34
Conclusion	34
Bibliography	34
Appendices	51
<i>Figure S1</i>	51

Figure S2	51
Table S1	52
Table S2	54
Table S3	56
Table S4	58
Statement of Expenditure	59
Statement of Contributors	61
Ethics Approval	62
Risk Assessments	63
DNA Extraction	63
Co-cultivation	71
Risk Assessment of An Activity Involving Deliberate Work with Pathogenic Microorganisms or Samples with Potential to Harbour Pathogenic Microorganisms	77

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List of Tables and Figures

Figure 1: Alpha diversity of the microalgal microbiomes grown on digestate (treatment 1 and 2) and the control medium over time (day 0, 4, and 7) a) rank transformed mean Shannon diversity, b) rank transformed Pielou's evenness, c) Chao1 richness. Error bars represent $\pm$ standard deviation (n=3). Curated within R (80).	17
Figure 2: Non-Metric Multi-Dimensional Scaling (NMDS) of the associated microbial communities of the digestate samples based on their Bray-Curtis distance matrix (beta diversity). Shapes indicate the treatments (1 = treatment 1, 2 = treatment 2, c = control) the samples were grown on and the colours indicate the days the samples were taken (n = 3). Curated using R (80).	18
Figure 3: Relative abundance of the bacterial classes in the microalgal microbiomes. Only classes with relative abundance greater than 1% across all the samples were included. Others were included in a group named "<1%". Curated using R (80).	19
Figure 4: The relative abundance of the 79 ASVs across all the microalgal microbiomes. The data has been log2 transformed. The last digit (1-3) of each column name indicates the replicate of each sample (n = 3). Curated within R using ComplexHeatmap (80).	21
Figure 5: The relative abundance of level 2 KEGG pathways in the microalgal microbiomes based on functional analysis of the amplicon data using Tax4Fun2. The last digit (1-3) of each column name indicates the replicate of each sample (n = 3). Curated using R (80).	23
Figure 6: The relative abundance of level 1 KEGG pathways in the microalgal microbiomes based on functional analysis of the amplicon data using Tax4Fun2. The last digit (1-3) of each column name indicates the replicate of each sample (n = 3). Curated using R (80).	24
Figure 7: Mean growth measurement (optical density (OD) at 750 nm) of co-cultivation treatments and <i>T. obliquus</i> control. Error bars represent $\pm$ standard deviation (n=3). Curated using R (80).	25
Figure 8: Mean a) growth rate per day b) final yield of all treatments, 8 co-cultivation treatments and 1 <i>T. obliquus</i> control treatment. One-way analysis of variance (ANOVA) was used to identify any significant differences between treatments ($p < 0.05$). Different lowercase letters indicate treatments that were significantly different from one another. Error bars represent $\pm$ standard deviation (n=3). Curated using R (80).	26
Table 1: Summary of Tukey's HSD post-test for the significant pairwise differences between final yields across the nine treatments (8 co-cultivation, 1 control).	26

Abbreviations

- **Qiime2**: Quantitative Insights into Microbial Ecology 2.
- **ASV**: Amplicon Sequencing Variant.
- **ANOVA**: Analysis of Variance.
- **PERMANOVA**: Permutational Multivariate Analysis of Variance.
- **NMDS**: Non-metric Multidimensional Scaling.
- **ALDeX2**: ANOVA-Like Differential Expression 2.
- **CLR**-transformed: Centred Log-Ratio transformed.
- **FDR**: False Discovery Rate.
- **KEGG**: Kyoto Encyclopedia of Genes and Genomes.
- **BLAST**: Basic Local Alignment Search Tool.
- **CCAP**: Culture Collection of Algae and Protozoa.

Introduction

There is an exceptional diversity of photosynthetic unicellular life that has major impacts on biogeochemical cycles (1). Photosynthetic unicellular life is extremely abundant and responsible for approximately half of the primary production globally (1). Microalgae are photosynthetic, unicellular microorganisms with rapid growth rates and wide-ranging tolerances to temperature, light intensity, pH, nutrient concentration, and salinity (2, 3). They can be cultivated using various media or within various environments including seawater, freshwater, and wastewater (4–6). Microalgae also possess distinctive, propitious attributes (7). For instance, their ability to produce stable isotopes and valuable lipids that can be utilised when manufacturing various commodities including health and cosmetic products (7). These attributes coupled with the robust, tolerant, and flexible nature of microalgae have resulted in these organisms being exploited for several uses within biotechnology and being excellent candidates for a greener, bio-based circular economy in the future (8).

While humans utilised microalgae over 2000 years ago, and research into microalgal biotechnology began in the mid-20th century, only recently have there been significant advances in the development of microalgal biotechnology (8–12). Both commercial and industrial research has been conducted to investigate their potential uses (7). Commercially, microalgae have been used within human health supplements and their pigments have been used to colour food, cosmetics, and pharmaceuticals (8, 13, 14). Industrially, the use of microalgae to derive biofuels, biofertilizers, and animal feed has been researched extensively (15–18). Currently, the incorporation of microalgae within bioremediation systems and bioreactors to treat wastewater is an area of particular interest (10, 11, 19, 20).

Poorly treated wastewater is one of the main threats to natural environments and drinking water worldwide (21). Municipal, agricultural, and industrial wastewater discharge often pollutes freshwater sources with large amounts of organic and inorganic contaminants including nitrogen and phosphorus compounds (22). When wastewater containing high concentrations of these compounds enters aquatic systems it causes eutrophication and promotes harmful cyanobacterial blooms (23). These blooms reduce the dissolved oxygen within aquatic environments (24, 25). This is caused by the death and decomposition of the cyanobacterial or algal blooms, and when the dissolved oxygen is reduced the fish and invertebrate species beneath the surface of the water cannot survive (25). Moreover, the blooms can prevent light from penetrating to the bottom of aquatic environments, consequently reducing the rate of photosynthesis of submerged plants attached to the substrate (24). This further reduces the dissolved oxygen concentrations, exacerbating the problem. These changes can significantly alter aquatic communities, and inefficient treatment and discharge of wastewater squanders valuable nutrients that could be recovered and recycled within microalgal wastewater treatment systems (26).

By incorporating microalgae within wastewater treatment systems, nitrogen or phosphorus compounds are recovered and the resulting microalgal biomass can be used elsewhere, thus contributing to a circular economy (10). As nitrogen and phosphorus compounds are vital for microalgal growth and are abundant in wastewater sources, microalgae often grow well within wastewater and can uptake these compounds that, in excess, are harmful to the environment (27). This process allows for the recycling of useful nutrients and makes the water more inert (28). The resulting microalgal biomass can be utilised as biofertilizers, manufactured as feed for agricultural and aquaculture industries, or used within other high-value products (10, 29). Despite the obvious benefits of using microalgae within wastewater treatment systems, there have been challenges in scaling up these systems robustly and they are not yet as efficient as traditional wastewater treatment systems (30).

Traditional wastewater treatment systems reduce the biological oxygen demand using activated sludge and primarily focus on removing suspended solids (31). These systems aim to remove or break down contaminants in wastewater to allow for the water to be disposed of safely or reused (22). While these systems remove satisfactory levels of nitrogen, phosphorus, and carbon compounds, they consume high levels of energy, require supplementary oxygen input, and some nutrients are often not fully recovered (30, 32). This results in the, mostly accidental, release of these nutrients into the environment (30). Moreover, the degradation capacities of these traditional systems are restricted, particularly when they encounter exceptionally high nutrient loads (33). If the scaling up and efficiency issues associated with microalgal wastewater treatment systems can be resolved, microalgal addition in these systems may provide a solution to the problems faced with traditional treatment methods. To resolve these issues, more research into what impacts microalgal growth needs to be conducted.

It is well known that specific strains of bacteria promote the growth of terrestrial plants, a process that has already been utilized and benefits industrial horticulture (34). Some of these plant-growth-promoting strains, such as *Azospirillum brasilense*, have also been shown to increase the growth of microalgae (35–37). More recently, studies have investigated how bacteria naturally associated with microalgae in the phycosphere impact microalgal growth (2, 38). The phycosphere is a microscopic chemically enriched region surrounding a microalgal cell where tight interactions occur between microalgae and other microorganisms (39–41). These interactions are mediated by exuded chemicals and there are various mechanisms involved in the relationships between microalgae and the other organisms within this environment (39, 41). Based on current research, the principal mechanisms involved in the relationship and interactions between microalgae and bacteria, are the exchanging of nutrients, cell-to-cell communication, and stimulation via chemical compounds (42–44). These mechanisms form the basis of symbiotic relationships between microalgae and bacteria.

Symbiotic relationships between microalgae and bacteria can take many forms and result in positive, negative, or neutral effects for microalgae (45). A mutualistic interaction results in benefits for the two or more organisms involved in the interaction (45). For example, when nitrogen-fixing bacteria provide microalgae with inorganic nitrogen for growth and, in exchange, microalgae supply the bacteria with fixed carbon (46, 47). Unlike mutualism, commensalism is a relationship where only one organism benefits from the relationship (47). An instance of commensalism is the relationship between *Chlamydomonas reinhardtii* and heterotrophic bacteria. *C. reinhardtii* utilises vitamin B₁₂ derived by heterotrophic bacteria, despite the bacteria not utilising the organic carbon provided by the microalga (48). Parasitism causes one organism to benefit at the expense of the other and often impacts the latter organism negatively (45). These relationships can change and switch to one another over time depending on nutrient concentrations, competition, and other environmental factors (45, 47). Consequently, it is vital to study and understand the relationships and effects of specific microalgae-bacteria interactions to fully benefit from and exploit them within biotechnology.

An example of these relationships being utilised within biotechnology is the use of parasitic relationships as a form of biocontrol (49). By implementing or introducing parasitic bacteria to a harmful algal bloom this could cause the bloom to diminish and prevent harm to the surrounding environment and wildlife (49). Another promising use of these interactions is that mutualistic relationships could be used to increase the efficiency and upscale microalgal wastewater treatment systems (28). Recent research has shown that mutualistic interactions between microalgae and bacteria increase the growth rate of microalgae by up to 70% (44, 47). Furthermore, it has been reported that this synergistic collaboration results in excellent nutrient recovery, reducing the potentially polluting discharge and allowing for the reuse of these nutrients within microalgal

biomass (44, 50). By identifying strains of bacteria that increase the growth rates of microalgae as well as increasing nutrient recovery, wastewater treatment systems could become more efficient and less polluting (28).

There are still significant research challenges when trying to integrate microalgae-bacteria consortia into wastewater treatment systems (28). However, identifying bacterial strains associated with microalgae and investigating the effects of specific strains on microalgal growth can be the first steps when aiming to recognise mutualistic beneficial relationships between microalgae and bacteria that can be utilised within wastewater treatment systems. One commonly used species in microalgal wastewater treatment systems is *Tetradismus obliquus* (previously referred to as *Scenedesmus obliquus*), a robust freshwater species (51, 52). *T. obliquus* biomass has been used to derive biofuel and biofertilizers, used within aquacultural feed, and developed as biosensors to detect harmful pollutants (53). The use of *T. obliquus* in wastewater treatment has been well researched (51–55). *T. obliquus* has been used previously due to its high growth rates, high tolerances, and efficiency at removing nutrients from wastewater (53, 55). Research has shown that this species removes nutrients with greater efficiency than the more commonly used microalga *Chlorella vulgaris* and can remove some pharmaceutical compounds that *C. vulgaris* is unable to (53, 55). *T. obliquus* also accumulates quickly in high ammonium environments and could potentially thrive in digestate or wastewater (56). These characteristics make this species an ideal candidate for use in wastewater treatment systems.

Some preliminary research has been conducted to investigate the microbiomes associated with specific algal species, including studies evaluating the microbes associated with *T. obliquus* (57–61). Generally, bacteria belonging to *Proteobacteria* and *Bacteroidota* phyla are the most common strains to form symbiotic relationships or be associated with microalgae (60–62). Bacterial strains belonging to these phyla can rapidly degrade organic matter, allowing for the direct transferral of organic carbon that microalgae provide (60). One co-cultivation study showed how the actinomycete, *Nocardia bhagyanarayanae* I-27 can increase *T. obliquus* growth and lipid production within 25% diluted digestate (63). While several studies have confirmed that *T. obliquus* grows faster with bacterial strains present rather than in axenic conditions, there has been little research reviewing the specific strains associated with *T. obliquus* and how these specific strains may impact the growth of this commercially important microalgal species (64). In one such study from 2015, Wang *et al.*, (64), investigated bacterial strains associated with *T. obliquus* and found that *Acidovorax facilis* increased the growth of *T. obliquus* by up to 24.8% which was the greatest increase out of five selected bacterial strains. However, Wang *et al.*, (64) reviewed co-cultivation within photoautotrophic systems rather than within bioremediation systems or on agricultural digestate, and therefore, the strains identified in Wang *et al.*, (64) may not be suited for use in microalgal wastewater treatment systems.

This study aimed to assess the bacterial communities of *T. obliquus* grown on three treatments with varying concentrations of agricultural digestate (one with no agricultural digestate, one with 50% f/2 media and 50% agricultural digestate and one treatment containing fully concentrated digestate), identify bacterial strains associated with *T. obliquus* during cultivation on agricultural digestate, and investigate how selected bacterial strains impact *T. obliquus* growth. To achieve these aims, the bacterial diversity within *T. obliquus* samples grown on agricultural digestate was analysed via DNA extractions and 16S rRNA amplicon sequencing. Co-cultivation studies of bacterial isolates from these samples were conducted to investigate how specific bacterial strains affect the growth of *T. obliquus*.

Currently, there is some discussion and disagreement when identifying the microalgal microbiome vs the co-occurring community. In this study the ‘microalgal microbiome(s)’ refer(s) to the bacterial

community within the samples which are assumed to be within the microalgal microbiome. Without further investigation this community cannot be identified as either the co-occurring community or the microalgal microbiome, however conclusions on those strains suitable for microalgal wastewater treatment systems are drawn through the community analysis of the samples grown on digestate and the co-cultivation studies.

Materials and Methods

Cultivation of microalgal samples for DNA extraction

The samples used for the first part of this study were obtained during a previous project (performed by Vittorio Lim, Mohamed Emran, Alla Silkina, and Eva Sonnenschein) investigating the effect of digestate, the liquid obtained from anaerobically digested animal manure, on *T. obliquus* growth. During July 2023, *T. obliquus* CCAP 276/46 was cultivated using three treatments: a control, treatment 1, and treatment 2. The medium for the control treatment was f/2 medium (Guillard's (F/2) Marine Water Enrichment Solution) (Sigma-Aldrich) prepared with dH₂O with no digestate added, containing 12 mg/L nitrogen (from NaNO₃). Treatment 1 had both f/2 medium (12 mg/L nitrogen (from NaNO₃)) and digestate (added to a total nitrogen content of ~40 mg/L; mostly containing NH₄ and digestate to provide a diluted digestate treatment). In treatment 2 the microalgae were cultivated solely on digestate at a total nitrogen content of ~40mg/L. The microalgae were cultivated in 20 L carboys at a light intensity of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, a day/night cycle of 16:8, 18°C aerated with CO₂ mixed with air (0.01%). Each treatment was run in triplicates and samples were taken on days 0, 1, 4, 7, 10, and 14 for analysis of total nitrogen, ammonium, nitrate, and phosphate content, optical density, and dry weight of biomass.

Due to the greatest shift in nitrogen content occurring in the first seven days, samples from days 0, 4, and 7 were used for the microalgal microbiome analysis, resulting in a total of 27 samples. 50 ml of sample were centrifuged, the supernatant discarded, and the pellet stored at -80°C until DNA extraction. The work described from here onwards / the DNA extraction onwards was completed by Alexandra Gimblett.

DNA extraction, and 16S rRNA gene amplicon library preparation

To investigate the bacterial diversity associated with *T. obliquus*, DNA was extracted using the MicroGEM prepGEM bacteria kit for 'DNA extraction from capsulated bacteria/colonies/biofilms' following the manufacturer's instructions with a slight modification (MicroGEM, Charlottesville, VA); the pellets were resuspended in 100 μl of 1X WASH+ buffer prior to DNA extraction rather than pipetting 400 μl of 1X WASH+ buffer into a 1.5 ml microcentrifuge tube and resuspending a small amount of a bacterial colony/biofilm. Post extraction the samples were kept at -26°C in a freezer to preserve the DNA. DNA concentration and quality were quantified using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, UK) and agarose gel electrophoresis.

For 16S rRNA gene amplicon library preparation, the V4 region of the 16S rRNA gene was amplified in a first PCR using the prokaryotic primer pair recommended by the Earth Microbiome project (the updated version of 515F – GTGYCAGCMGCCGCGGTAA and 806R – GGACTACNVGGGTWTCTAAT) (65–68), followed by the addition of barcodes to the amplicons in a second PCR using the Nextera XT Index Kit (Illumina, UK). In detail, the DNA samples were diluted to 1:1 (20 μl of sample and 20 μl of PCR grade water) to dilute potential PCR inhibitors. The protocol followed the Illumina Metagenomic Sequencing Library Preparation guide (69) with slight modifications to the amounts of primers, water, Platinum II PCR master mix (MM) (Thermo Fisher Scientific, UK), and DNA. For the first PCR, the amounts of each component in a total reaction volume of 20 μl were as follows: 10 μl Platinum II PCR

MM, 0.4 µl forward primer 515F (Invitrogen, Thermo Fisher Scientific, UK), 0.4 µl reverse primer 806R (Invitrogen, Thermo Fisher Scientific, UK), 7.2 µl PCR grade water (Thermo Fisher Scientific, UK) and 2 µl genomic DNA. PCR amplification was performed with an initial denaturation at 95°C for 3 minutes, followed by 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, then a final elongation at 72°C for 5 minutes. For the second PCR amplification, 2 µl of PCR product from the first amplification were mixed with 10 µl of Platinum II PCR MM, 7.5 µl of PCR grade water and 1 µl of each Nextera XT Index primer (Nextera XT Index 1 Primers (N7XX), Nextera XT Index 2 Primers (S5XX); Illumina, UK) with a total reaction volume of 21.5 µl. The second PCR amplification was performed with an initial denaturation at 95°C for 3 minutes, followed by 8 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, then a final elongation at 72°C for 5 minutes.

Following the second PCR reaction, amplicons were subjected to gel electrophoresis on a 2% agarose gel to identify samples with stronger or weaker bands. Samples were mixed into two pools, one with high concentrations of DNA (stronger bands) and the other with low concentrations of DNA (weaker bands), and then purified using Agencourt Ampure XP beads (Beckman Coulter) following the manufacturer's protocol. A qPCR was carried out using the Library Quant Kit (NEBNext, Illumina) following the manufacturer's protocol to determine the average concentrations of each pool. Using the results from this qPCR the pools were combined in equal concentrations and submitted to sequencing on an Illumina MiSeq at the sequencing core facility at Swansea University by Matt Hitchings.

Bioinformatic and statistical analysis of the microalgal microbiomes

Bioinformatic analysis

Bioinformatic analysis was conducted using Qiime2 (v2024.02, (70)) (workflow shown in Figure S1) on the Galaxy interface (71). Using DADA2 within Qiime2, all reads were truncated to 258 bp (forward reads) and 188 bp (reverse reads) based on overall quality scores, and the first 20 bp were trimmed to remove the primers and any potential contamination from adapters (70, 72). Following this, the reads were denoised, merged, and allocated into actual sequence variants (ASVs) using DADA2 (72). Taxonomic assignment was completed using the Greengenes2 database (v2022.10, (73)) and a pre-trained classifier from 515F 806R region of 16S rRNA gene sequences provided on the Qiime2 docs (73, 74). ASVs classified as mitochondria and chloroplasts were filtered out and those with more than 100 reads were retained in the final ASV table to allow for downstream analysis. Analysis of the feature table and alpha and beta rarefaction plots were used to confirm that the value of 34,982 sequence counts was an appropriate sampling depth. This ultimately did not exclude any samples. No rarefying was conducted. Alpha and beta diversity metrics were calculated to show the similarities and differences within and between the bacterial communities found in the samples, specifically Pielou's evenness (75), Shannon diversity (76), Chao1 richness (77), Bray-Curtis distance (78).

Alpha and Beta diversity of the microalgal microbiomes

R was used to statistically analyse and visualise the alpha and beta diversity metrics to determine any differences between the bacterial communities (78). Packages 'qiime2R' and 'readr' were used to import and read Qiime2 files and metadata files into R (79, 80).

The Shannon diversity and Pielou's evenness were rank transformed to meet the ANOVA assumptions of normality and homogeneity of variance (Shapiro-Wilk test, Bartlett test) (81, 82). For the Shannon diversity and Pielou's evenness, a one-way ANOVA was performed to determine any significant differences between the samples' species diversity or evenness followed by Tukey's HSD

post hoc tests to identify which, if any, specific samples differed. No transformations of the Chao1 richness made the data meet the assumptions of an ANOVA, so a Kruskal-Wallis test was used instead followed by a Dunn test (with Bonferroni as the p adjustment method) to determine any differences between specific samples (83, 84). Bar plots were curated using R to visualise the differences in the individual samples' average Shannon diversity, Pielou's evenness, and Chao1 richness (80).

Beta diversity, based on Bray-Curtis distance, was visualised using nonmetric multidimensional scaling (NMDS) ordination and analysed with outputs produced by Qiime2 (70). These outputs were used to create a phyloseq in R with the packages 'phyloseq', 'dplyr', 'tidyr', 'ape', and 'stringr' (80, 85–89). The NMDS visualisation was curated based on Bray-Curtis distance within R using the phyloseq and the function 'ggplot', which belongs to the 'tidyverse' package (70, 80, 90). Multivariate tests of permutational analysis of variance (PERMANOVA) were performed within R using 'Adonis2', belonging to the 'vegan' package, with 999 permutations to determine if the bacterial communities within each microalgal microbiome sample were statistically different from one another (91, 92). The 'betadisper' function within the 'vegan' package was used in R to confirm any significant differences presented from the PERMANOVA analysis were due to differences between sample groups (the three separate treatments sampled on the three sampling days) rather than dispersions between replicate samples within the same group (91–93).

Abundance and functional analysis of the microalgal microbiomes

The presence and abundance of taxonomic class was used to provide a general overview of the bacterial community compositions present within the microalgal microbiome samples. Prior to visualisation, a phyloseq was created using packages 'phyloseq', 'dplyr', 'tidyr', 'ape', and 'stringr' (85–89). Using this phyloseq a bar graph showing the relative abundance of each class within each sample was curated using 'ggplot' within the 'tidyverse' package in R (80, 90). Only classes with greater than 1% relative abundance within one sample were selected for visualisations. The remaining classes were grouped as "<1%". To understand the differential abundance of the ASVs across days and treatments, ANOVA-Like Differential Expression (ALDEx2) was used within R (80, 94). ALDEx2 has been identified as one of the most reliable and consistent methods to analyse differential abundance (95). The 'aldex.glm' function was used after the data was CLR-transformed to provide a more compositionally coherent inference of any ASVs that were differentially abundant between days and treatments. The false discovery rate (FDR) was set to Benjamini-Hochberg to adjust for multiple testing. A heatmap showing the relative abundance of the ASVs across all samples was curated using the 'pheatmap' function within the 'ComplexHeatmap' package in R (80, 96).

Functional analysis of the ASVs was performed using Tax4Fun2 (v2_1) which is based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (97). Tax4Fun2 was selected to predict functional profiles as it has higher accuracy than other tools such as PICRUST and Tax4Fun and the predictions made are often more robust (97). Tax4Fun2 analysis was completed within R (80). The 'runRefBlast' function within the Tax4Fun2 package was used to run a BLAST search using the reference database (v2_1) provided on GitHub and the filtered ASV table and sequences produced using Qiime2 (70, 97). The 'makeFunctionalPrediction' function was then used to make functional predictions (97). To determine any differences in the abundance of varying level 2 KEGG pathways, Kruskal-Wallis tests were used followed by Dunn tests (with Bonferroni as the p adjustment method) to determine any differences between specific samples (83, 84). A bubble plot was curated to show the relative abundance of each functional gene pathway at KEGG level 2 within the samples using the 'reshape' and 'tidyverse' packages (90, 98). A heatmap showing the relative abundance of the top 50

most abundant functional gene pathways at KEGG level 1 within each sample was curated using the 'pheatmap' function within the 'ComplexHeatmap' package in R (80, 96).

Co-cultivation of bacterial isolates and *T. obliquus*

During the initial cultivation of *T. obliquus* on digestate, bacterial strains were isolated on LB/10 agar (Luria-Bertano medium, Fisher Scientific, UK) from the liquid *T. obliquus* samples and taxonomically identified using partial 16S rRNA gene sequencing using the primer pair 27F and 1492R (isolation and PCR performed by Vittorio Lim and Shirin Naserikarimvand). Eight unique bacterial strains were chosen for a co-cultivation experiment with *T. obliquus*. These strains were chosen based on if they were hazardous, if they had been used within laboratory based microalgal wastewater treatment systems previously or if they had been found in less tolerable environments. According to taxonomic comparison to the BLAST 16S rRNA database, the eight bacterial strains were closest related to: *Microbacterium algeriense* strain G1 (Percent Identity – 99.81%), *Brevundimonas aurantiaca* strain CB-R (99.82%), *Microbacterium oxydans* strain DSM 20578 (99.56%), *Solibacillus silvestris* strain HR3-23 (99.91%), *Pseudomonas fildesensis* strain KG01 (99.63%), *Sphingobium xenophagum* strain BN6 (99.72%), *Pseudomonas laurentiana* strain GSL-010 (99.02%), and *Bacillus toyonensis* strain BCT-7112 (99.83%). Each strain was restreaked from a cryostock (maintained at -80°C) on LB/10 agar and incubated at 25°C for 4 days. Bacterial strains were pre-cultured by inoculation of a single colony into 2 mL of LB/10 in 15 mL falcon tubes and incubated overnight at 25°C and 220 rpm.

An axenic culture of *T. obliquus* CCAP 276/6B was obtained from the Culture Collection of Algae and Protozoa, inoculated into f/2 medium at a ratio of 1.5:100 and kept in a shaking incubator at 18°C, 100 rpm, and with a constant light level of 39 $\mu\text{mol ms}^{-2} \text{s}^{-1}$ for 3 days. To check the axenic status of the culture, 100 μL of the axenic liquid culture was plated onto an LB/10 agar plate and incubated at 18°C for 3 days.

The co-cultivation of the eight bacterial strains and *T. obliquus* was set up in a sterile 96 well plate (Nuclon™ Delta Surface, Thermo Fisher Scientific) with 200 μL of sample per well. The bacterial pre-cultures were diluted 1:100 in f/2 prior to inoculation. As the algal pre-culture was already adequately dilute, (optical density/absorbance at 750 nm was <0.09) it was not diluted. 180 μL of algal pre-culture and 20 μL of diluted bacterial pre-culture or f/2 control were added per well. Additionally, 200 μL of f/2 media was used as a blank and 20 μL of each diluted bacterial pre-culture bacterial strain or algal pre-culture was inoculated into 180 μL f/2 to act as controls. All 18 setups (8 co-cultivations, 1 media control, 1 microalgal control, and 8 bacterial controls) were run in triplicates (Figure S2).

Initially, the samples were incubated at 18°C, with a constant light level of 39 $\mu\text{mol ms}^{-2} \text{s}^{-1}$, and at 100 rpm. After 24 hours, the plate was incubated at 18°C, with a constant light level of 63 $\mu\text{mol ms}^{-2} \text{s}^{-1}$, and at 400 rpm, for seven more days. This change was made as the liquid within the wells was not being sufficiently circulated and therefore not aerating at 100 rpm and light conditions were more favourable in the second incubator. Absorbance at 750 nm was measured daily in a spectrophotometer (SPECTROstar^{Nano}, BMG Labtech). The measurements were stopped after 8 days as this was deemed to be the time that the co-cultivation samples growth had slowed and any measurements beyond this may have been misleading due to any evaporation. The co-cultivation treatments with the highest and lowest absorbance were re-streaked onto LB/10 agar to check that no contamination had occurred. These plates were checked for five days after inoculation to ensure that no bacteria, other than the intended bacterial isolates, were present with the microalgae.

The co-cultivation treatments and microalgal control were carried forward for statistical analysis after confirmation that the media and bacterial controls showed no unexpected growth based on their

final yield values. This was verified by analysis of variance (ANOVA) comparing the final yields of the bacterial controls and the co-cultivation treatments to ensure they were significantly different ($p < 0.05$).

The absorbance measurements were used as the measurement of algal growth. This was visualised with a line graph using 'ggplot' within the 'tidyverse' package and the 'qiime2R' package in R (79, 80, 90). The growth rate between days 2 and 4 (where there was exponential growth) for each treatment was calculated using the following formula: $\mu = (\log_{10}N - \log_{10}N_0)/(t - t_0)$ where 'N' is the day 4 optical density reading, 'N₀' is the day 2 optical density reading, 't' is day 4 and 't₀' is day 2. The final yield was calculated by subtracting the day 0 absorbance value from the day 8 absorbance value for each treatment. The average growth rates for each treatment and the average final yields for each treatment were compared using ANOVA analysis and TukeyHSD post-hoc tests to determine any statistical differences between treatments. Bar graphs (with standard deviation as the error) were used to visualise this data and were curated within R (80).

Results

Nutrient information, collected by previous researchers, for the microalgal microbiome samples

Other researchers collected environmental data using the microalgal microbiome samples used in the present study. The nutrient concentrations, including the total nitrogen levels (mg/L), ammonia levels (mg/L), phosphate levels (mg/L), and nitrate levels (mg/L), the optical density (750nm), and the dry weight of the samples were measured. The fully concentrated digestate had the highest levels of ammonia and phosphate, the f/2 medium had the lowest, and the mixture of f/2 medium and digestate had a relatively moderate level of both nutrients on all three sampling days. Both these nutrients reduced in concentration over the 7 days in all treatments (Table S4).

Analysis of sequence data

A total of 3,777,974 reads were present prior to filtering. After filtering, denoising and merging the total reads was reduced to 2,376,450 and assigned to ASVs. Following the filtering out of ASVs containing less than 100 reads and taxonomic analysis, 79 ASVs remained. All the ASVs were assigned to *Bacteria*. All samples contained more than 34,900 reads and therefore, no samples were excluded due to low read counts.

Bacterial alpha diversity of the microalgal microbiomes

Diversity measures for these samples were similar for Shannon diversity and Pielou's evenness (Figure 1a, 1b). The control samples, which contained no digestate, had higher Shannon diversity and Pielou's evenness than at least one of the treatments containing digestate (treatments 1 and 2) on all three sample collection days. On days 0 and 7 the control treatment had higher species diversity and evenness than treatments 1 and 2. Notably, on day 4, treatment 1 had a higher species diversity and evenness when compared to both the control treatment and treatment 2.

One-way ANOVAs of the rank transformed Shannon diversity and Pielou's evenness indicated that there were significant differences between the samples' bacterial diversity and significant differences between the samples' bacterial evenness (Shannon diversity: $F_{8,18} = 2.608$, $p = 0.044$; Pielou's evenness: $F_{8,18} = 2.878$, $p = 0.030$). A Tukey's HSD post hoc test was used to determine which specific samples were significantly different. Despite the significant results from the ANOVAs, Tukey's HSD showed that no samples (rank transformed) Shannon diversity or Pielou's evenness were significantly different from one another. This implies that there may be significant differences between the samples' bacterial diversity and significant differences between the samples' bacterial evenness, yet

it cannot be determined between which specific samples there is a significant difference for both diversity metrics.

All samples appeared to have similar levels of Chao1 species richness, with day 0, treatment 1 having the lowest bacterial richness and day 7, treatment 2 having the highest bacterial richness (Figure 1c). A Kruskal-Wallis test of the Chao1 richness indicated that there was no significant difference between the samples' bacterial richness ($\chi^2(8) = 14.853$, $p = 0.062$) further suggesting that all samples had a similar number of bacterial species. A Dunn-Bonferroni post-hoc test confirmed that no samples had significantly different bacterial species richness compared to the others.

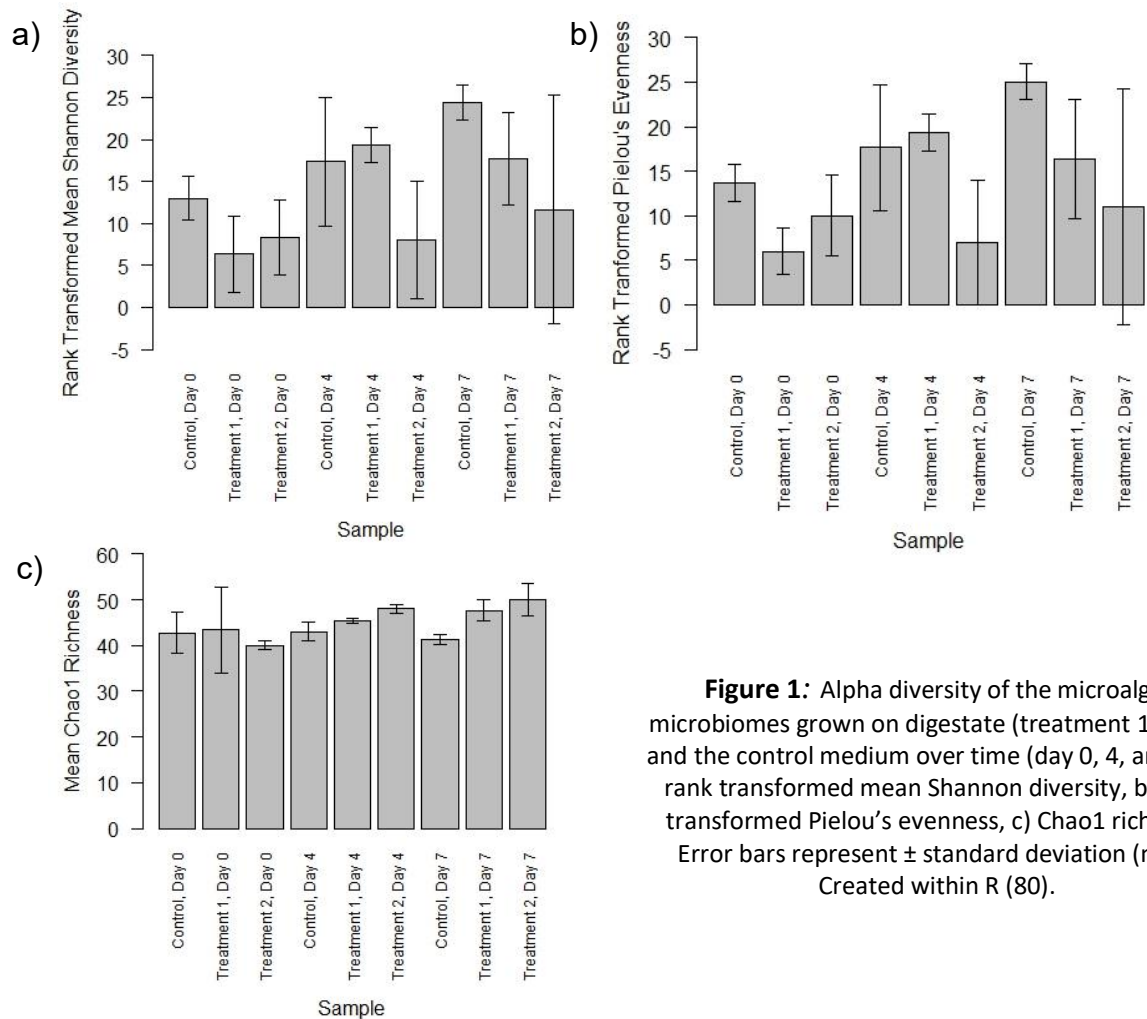


Figure 1: Alpha diversity of the microalgal microbiomes grown on digestate (treatment 1 and 2) and the control medium over time (day 0, 4, and 7) a) rank transformed mean Shannon diversity, b) rank transformed Pielou's evenness, c) Chao1 richness. Error bars represent ± standard deviation (n=3). Created within R (80).

Bacterial beta diversity of the microalgal microbiomes

Overall, the NMDS implies that, after day 0, the bacterial communities grown on different treatments tend to be separate from one another (Figure 2). This is particularly evident on day 7 (Figure 2, blue) where all three treatments were found in different data spaces within the NMDS plot. PERMANOVA analysis confirmed that there was a significant difference between samples when accounting for both the treatment the samples were cultivated on (control, treatment 1, or treatment 2) and the day the samples were taken (0, 4, or 7) (pseudo- $F_{8,18} = 8.247$, $p = 0.001$). A beta dispersion test verified that the significant difference between samples identified by PERMANOVA analysis was due

to dispersions between samples rather than dispersions between sample replicates/replicates within the same sample group (betadisper- $F_{8,18} = 1.765$, $p = 0.151$). This confirms that the differences witnessed between bacterial community structures are likely due to the levels of digestate within the treatments and succession over time.

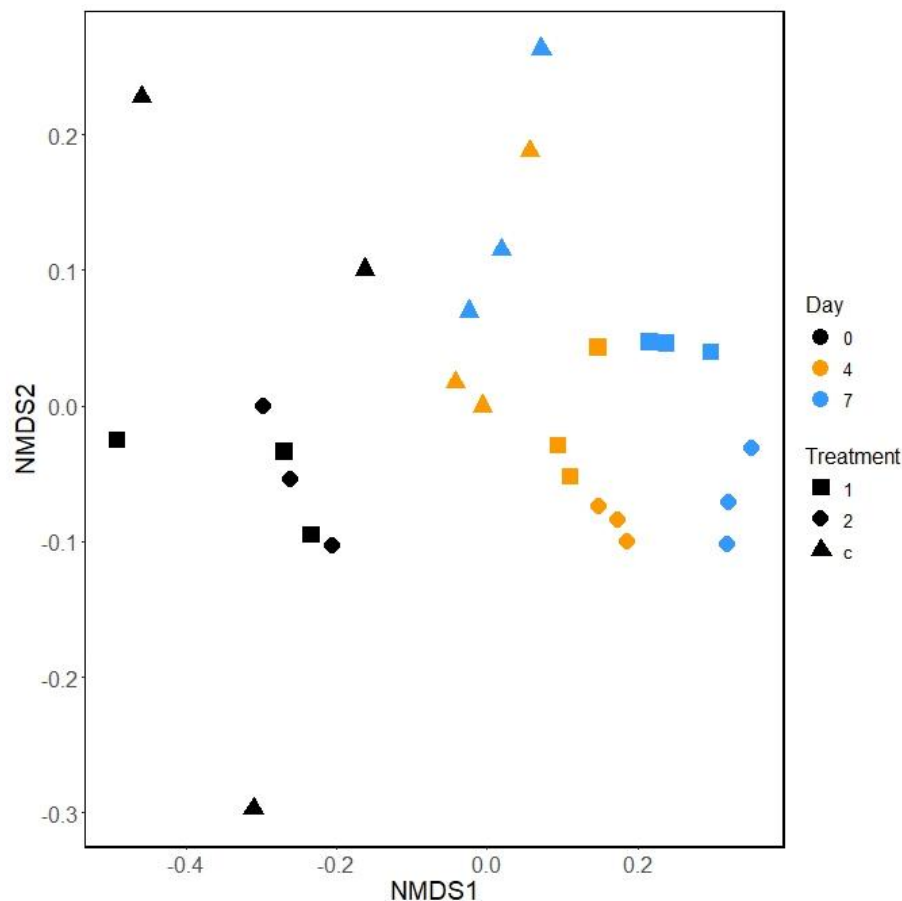


Figure 2: Non-Metric Multi-Dimensional Scaling (NMDS) of the associated microbial communities of the digestate samples based on their Bray-Curtis distance matrix (beta diversity). Shapes indicate the treatments (1 = treatment 1, 2 = treatment 2, c = control) the samples were grown on and the colours indicate the days the samples were taken ($n = 3$). Created using R (80).

Abundance of bacterial strains within the microalgal microbiomes

Ten taxonomic bacterial classes were found in all microalgal microbiome samples overall, with six of those, namely *Alphaproteobacteria*, *Cyanobacteriia*, *Bacteriodia*, *Bacilli*, *Actinomycetia*, and *Gammaproteobacteria*, having a relative abundance of more than 1% across all the samples (Figure 3). The relative abundance of the taxonomic classes within the bacterial communities appeared different depending on the day and treatment. *Alphaproteobacteria* dominated the bacterial community across all samples, totalling at least 40% of the overall relative abundance. This class was most abundant in samples collected on day 0 and within the control treatments across the three days. However, the abundance of *Alphaproteobacteria* decreased in treatments 1 and 2 over time. *Gammaproteobacteria* was the second most abundant taxonomic class across the bacterial

community within all samples, totalling 24% relative abundance. While *Gammaproteobacteria* was present in low abundance in the samples collected on day 0, its abundance increased dramatically in the following days especially within the treatment 1 and treatment 2 replicates. *Bacilli* was most abundant in treatments 1 and 2 on day 0, ranging from 27%-49% relative abundance. After day 0 *Bacilli* was only present in two treatment 1 replicates, all treatment 2 replicates on day 4, and one treatment 2 replicate on day 7 at very low abundances. Both *Bacteroidia* and *Cyanobacteriia* were present in all treatments across the three days with fluctuating abundances. *Bacteroidia* was most abundant on day 7 within the control and treatment 1 replicates. *Cyanobacteriia* was most abundant within the day 4 treatment 1 and treatment 2 replicates. *Actinomycetia* was present in exceptionally low abundances in every sample, with values ranging from 0.02%-2%, and was the least abundant class while still having a relative abundance greater than 1% across all samples. Altogether, differences in the bacterial community composition depended on the day and treatment of the samples, with more obvious differences when comparing the communities sampled on day 0 with those collected on the subsequent two sampling time points.

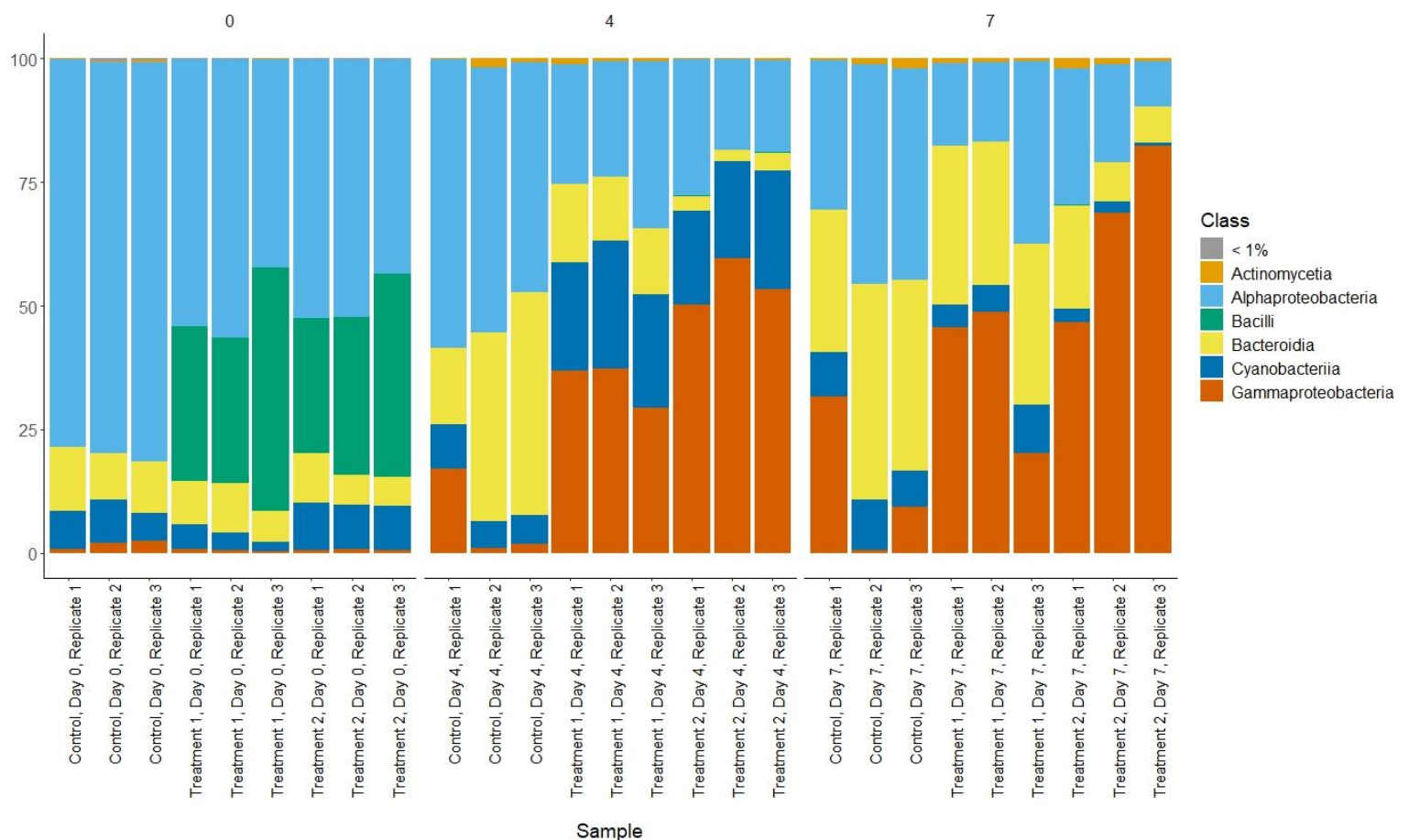


Figure 3: Relative abundance of the bacterial classes in the microalgal microbiomes. Only classes with relative abundance greater than 1% across all the samples were included. Others were included in a group named "<1%". Created using R (80).

A total of 79 ASVs were identified across the microalgal microbiomes (Figure 4). Three groups of samples appeared to have similar abundances of the ASVs. Samples collected on day 0 had similar

abundances of the ASVs, treatments 1 and 2 collected on both days 4 and 7 had similar abundances of the ASVs, and the control samples collected on days 4 and 7 had similar abundances of the ASVs. These three clusterings of samples show how the abundances of the ASVs changed over the days and differed depending on if there was digestate present within the treatment after day 0. The ASV identified as '*Brevundimonas* sp.' had the greatest overall relative abundance when evaluating the ASVs, totalling 18% of the ASV relative abundance. Notably, ALDEx2 analysis revealed that the abundances of *Comamonas*_F_589250 sp., *Pseudomonadaceae* sp., *Burkholderiaceae*_A_592522 sp., and *Sphingopyxis* sp. were significantly higher in treatments 1 and 2 on days 4 and 7 when compared to their abundance within the other samples ($p < 0.05$), with *Comamonas*_F_589250 sp., and *Pseudomonadaceae* sp., having high overall abundances (10% and 9% relative abundance respectively) (Table S2). Furthermore, ALDEx2 analysis revealed that SIO2C1 sp010672925 was found in significantly greater abundance in treatments 1 and 2 on day 4, *Runella zeae* was found in significantly greater abundance in treatment 1 on both days 4 and 7, *Pigmentiphaga* sp. was found in significantly greater abundance in treatment 2 on both days 4 and 7, and *Reyranelia aquatilis* was found in significantly greater abundance in treatments 1 and 2 on day 7 when compared to their abundance within the other samples ($p < 0.05$)(Table S2). These eight ASVs were the only strains with obviously and significantly higher abundances within at least one treatment containing digestate on days 4 and/or 7 when compared with the control and day 0 samples.

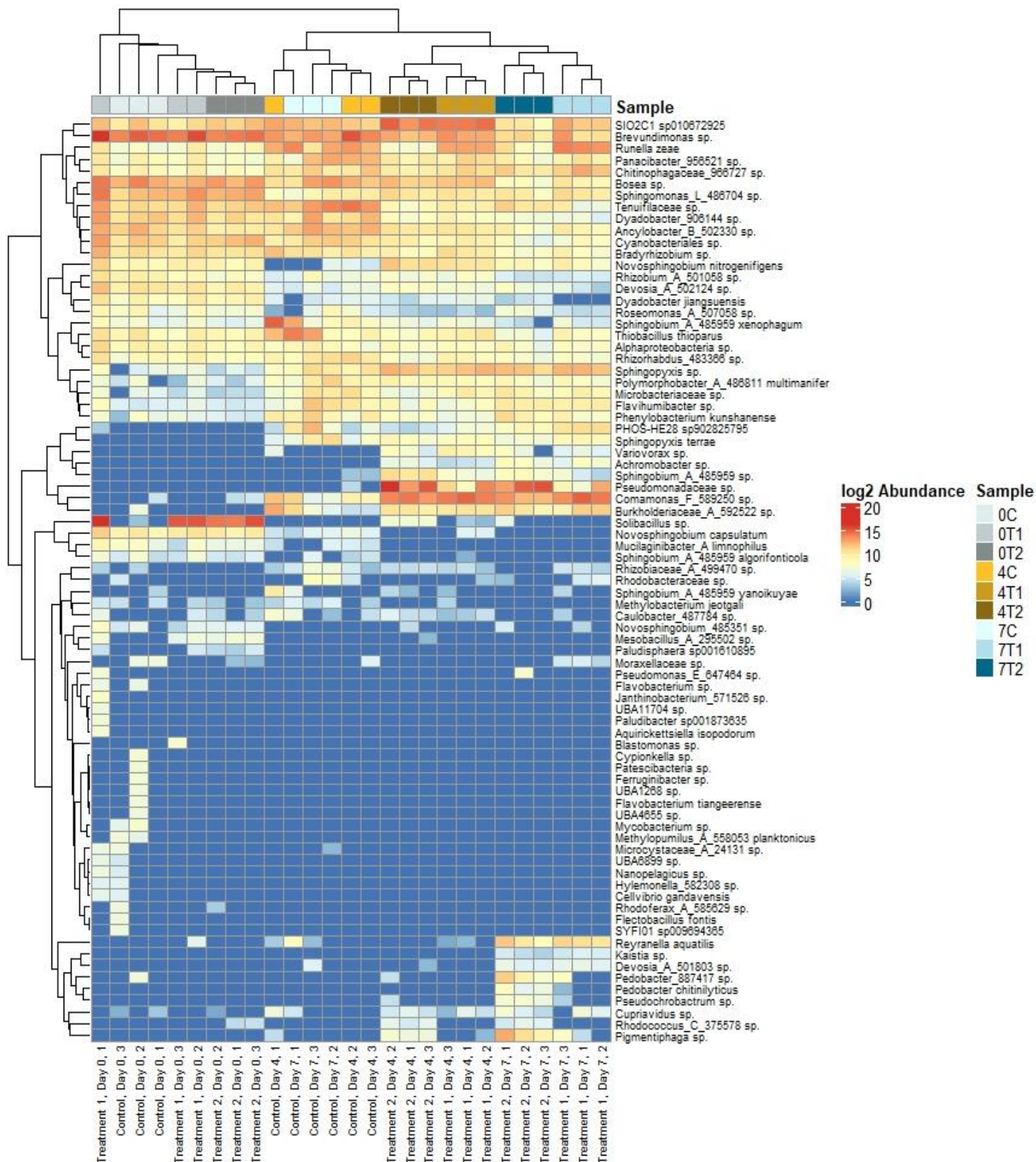


Figure 4: The relative abundance of the 79 ASVs across all the microalgal microbiomes. The data has been log2 transformed. The last digit (1-3) of each column name indicates the replicate of each sample (n = 3). Created within R using ComplexHeatmap (80).

Functionality analysis of the bacterial communities within the microalgal microbiomes

Tax4Fun2 was used to predict the functional profiles of the 27 microalgal microbiome samples based on the amplicon data. The bacterial communities within the samples were mainly associated with metabolism level 3 KEGG pathways (73%), with the remaining abundance composed of environmental information processing (11%), cellular processes (8%), human diseases (4%), genetic information processing (3%) and organismal systems (1%) pathways. A total of 45 level 2 KEGG pathways were identified (Figure 5). When evaluating all the bacterial communities within the samples, the level 2 KEGG pathways with relatively high abundances included: global and overview maps (36%), carbohydrate metabolism (9%), amino acid metabolism (8%), membrane transport (6%), signal transduction (5%) and cellular community – prokaryotes (5%). When comparing the varying days and treatments of the samples, the relative abundance of each level 2 KEGG pathway was comparably similar between the samples besides a few exceptions. According to Kruskal-Wallis tests and subsequent Dunn-Bonferroni post-hoc testing, there were only significant differences between two samples for four of the most abundant level 2 KEGG pathways (global and overview maps, amino acid metabolism, membrane transport, cellular community – prokaryotes), with the remaining two (carbohydrate metabolism, signal transduction) not having any relative abundance differences between samples (Table S1). Furthermore, the majority of level 2 KEGG pathways either had no significant differences between any specific samples or there was only a significant difference between 2 samples out of the 9. This suggests that the majority of samples had uniform abundances of functional genes and therefore, similar functional profiles. All the level 2 KEGG pathways and the samples with significantly different abundances of those pathways can be found in Table S1.

The frequently identified subsystem of the most abundant level 2 KEGG pathway (global and overview maps) was metabolic pathways, followed by microbial metabolism in diverse environments, biosynthesis of secondary metabolites, and biosynthesis of antibiotics. Metabolic pathways was the most abundant level 1 KEGG pathway overall (Figure 6). There appeared to be four clear groups of samples that had similar abundances of level 1 KEGG pathways. Treatment 2, collected on day 7, and treatments 1 and 2 collected on day 4 appeared to have similar abundances of the level 1 KEGG pathways. The control treatment collected on day 7, two replicates of the control treatment collected on day 4, one replicate of the control treatment collected on day 7, and one replicate of treatment 1 collected on day 0 showed similar abundances of level 1 KEGG pathways. Two replicates of the control treatment collected on day 0, two replicates of the control treatment collected on day 7, and one replicate of treatment 2 collected on day 0 had similar abundances of level 1 KEGG pathways. Finally, two replicates of both treatment 1 and 2 collected on day 0, and one replicate of each control collected on days 0, and 4 appeared to have similar abundances of level 1 KEGG pathways. These clusters do not appear to show any obvious trends regarding the impact of the various treatments and time on the abundances of level 1 KEGG pathways.

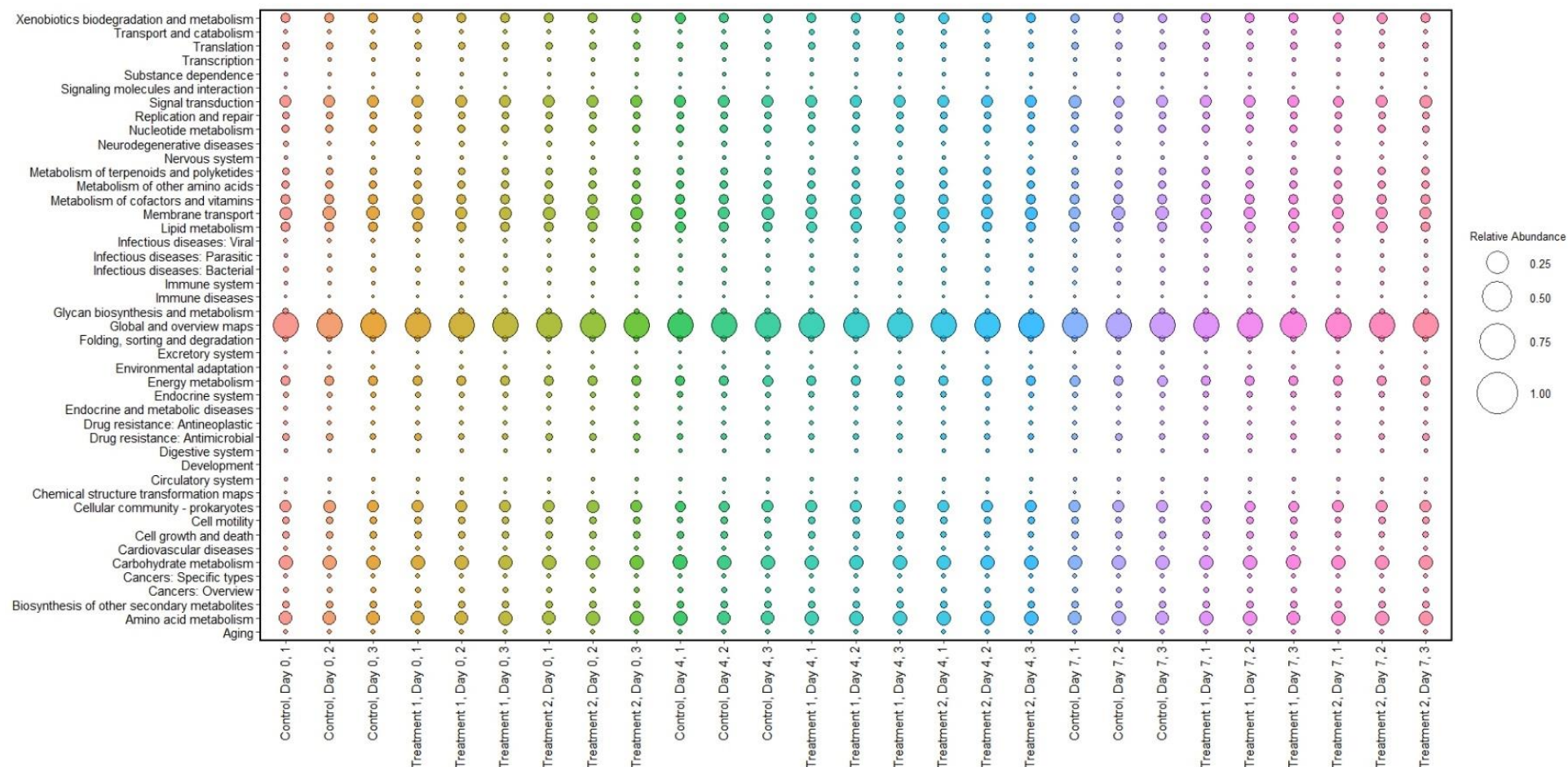


Figure 5: The relative abundance of level 2 KEGG pathways in the microalgal microbiomes based on functional analysis of the amplicon data using Tax4Fun2. The last digit (1-3) of each column name indicates the replicate of each sample (n = 3). Created using R (80).

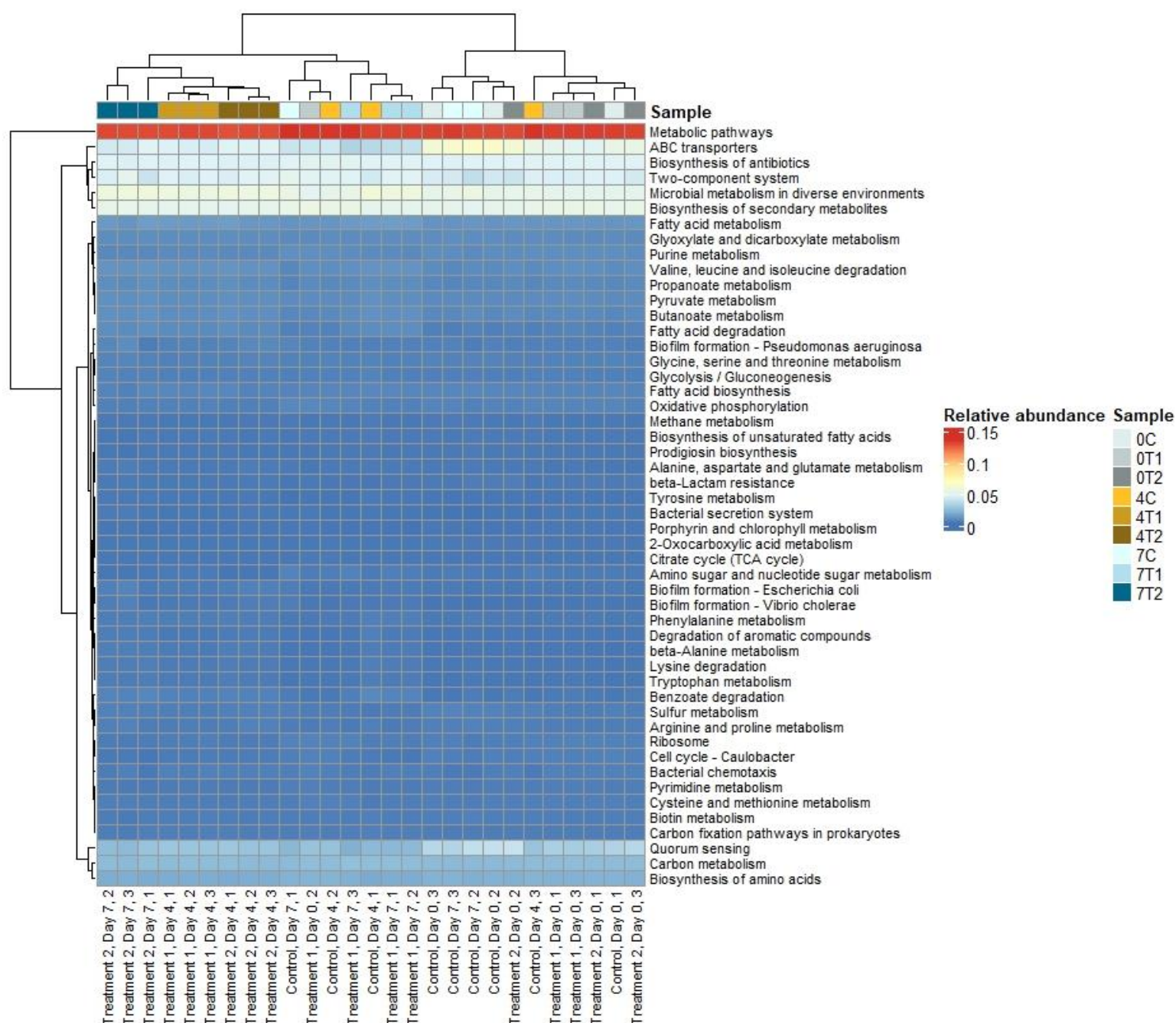


Figure 6: The relative abundance of level 1 KEGG pathways in the microalgal microbiomes based on functional analysis of the amplicon data using Tax4Fun2. The last digit (1-3) of each column name indicates the replicate of each sample (n = 3). Created using R (80).

Co-cultivation of 8 different bacterial strains with *T. obliquus*

The eight co-cultivation treatments clearly differed from the *T. obliquus* monoculture (control). This indicates that the addition of bacteria clearly increased the growth of *T. obliquus* as ANOVA comparisons of the co-cultivation treatments and bacteria controls confirmed that the increased growth was not mistaken for bacterial cell growth (see Methods). In the co-cultivation treatments, *S. silvestris* caused the highest optical density on day eight, and *P. fildesensis* caused the lowest. Overall, the *T. obliquus* control caused the lowest optical density indicating there was the least growth in this treatment. To further assess the impacts of the bacterial strains on *T. obliquus* growth, the growth rates and final yields of each treatment were calculated and compared.

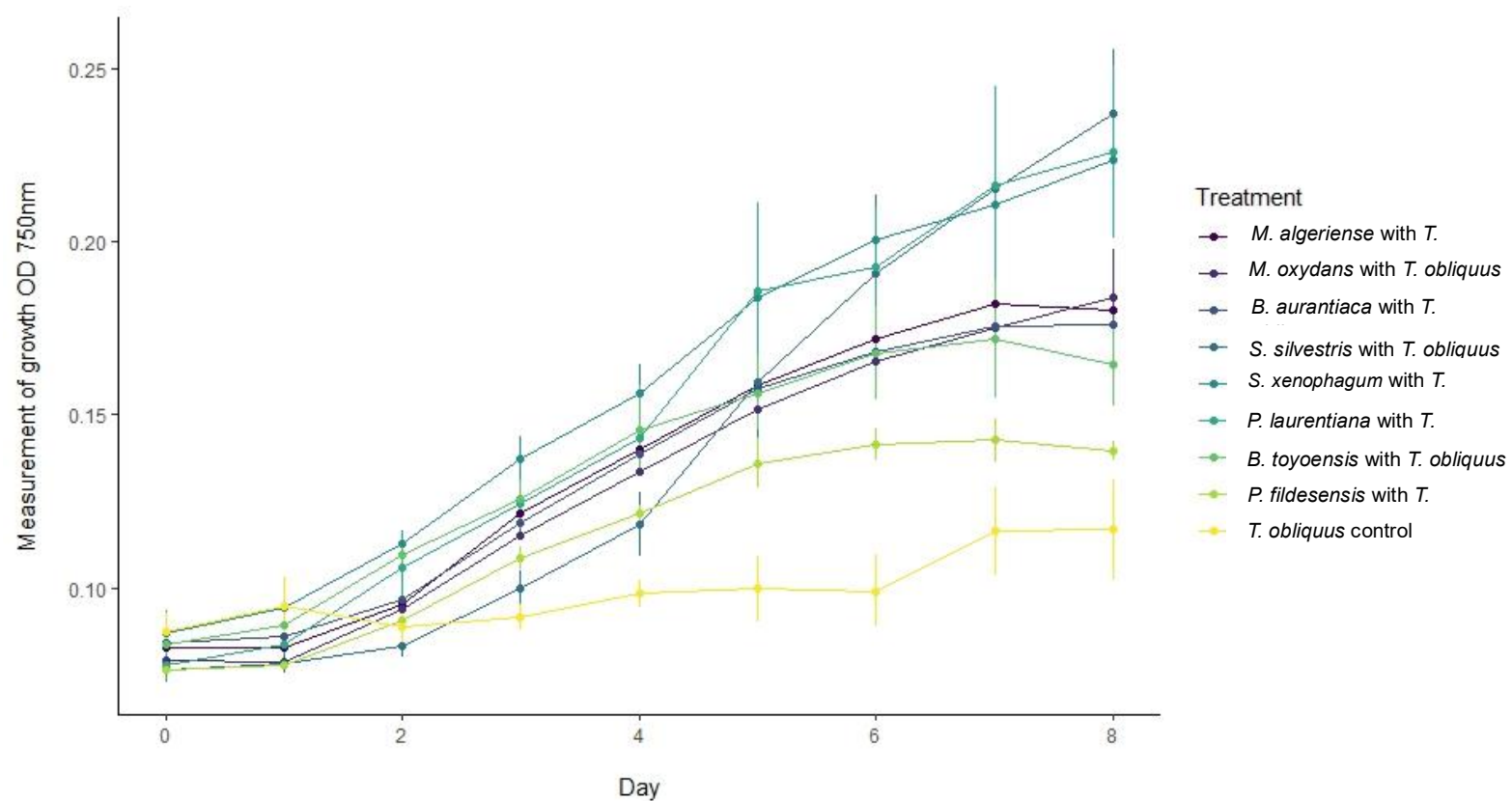


Figure 7: Mean growth measurement (optical density (OD) at 750 nm) of co-cultivation treatments and *T. obliquus* control. Different colours indicate different treatments. The measurements were taken over 8 days. Error bars represent $\pm$ standard deviation ($n=3$). Created using R (80).

A one-way ANOVA indicated that there were significant differences between the growth rates of treatments ($F_{8,18} = 3.407$, $p = 0.015$). To analyse the specific significant differences between treatments a Tukey's HSD post-hoc test was performed. No co-cultivation treatment's growth rate was significantly different from another. Four of the eight co-cultivation treatments' growth rates were significantly different from the *T. obliquus* control's growth rate. These included the co-cultivation treatments with *S. silvestris* ($p = 0.027$), *B. aurantiaca* ($p = 0.016$), *M. oxydans* ($p = 0.023$), and *M. algeriense* ($p < 0.01$) inoculated with *T. obliquus* (Figure 8a).

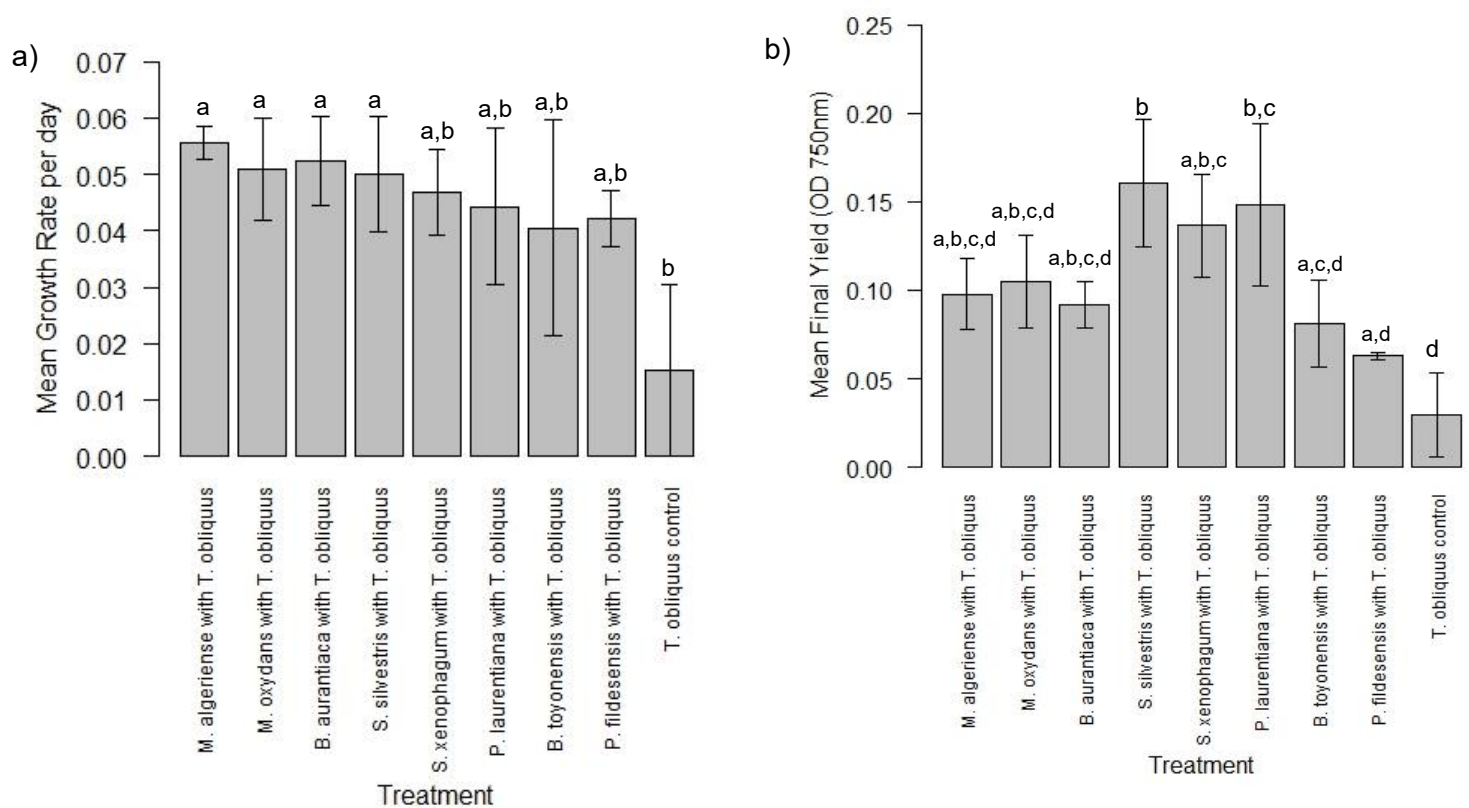


Figure 8: Mean a) growth rate per day b) final yield of all treatments, 8 co-cultivation treatments and 1 *T. obliquus* control treatment. One-way analysis of variance (ANOVA) was used to identify any significant differences between treatments ($p < 0.05$). Different lowercase letters indicate treatments that were significantly different from one another. Error bars represent $\pm$ standard deviation ($n=3$). Created using R (80).

As with the growth rate, a one-way ANOVA illustrated that there were significant differences between the final yields of the treatments (Figure 8b) ($F_{8,18} = 7.138$, $p < 0.001$). A Tukey's HSD post-hoc test was performed to assess the specific significant differences between treatments. *S. silvestris*, *S. xenophagum*, and *P. laurentiana* had significantly higher final yields from the *T. obliquus* control and both *S. silvestris* and *P. laurentiana*'s final yields were significantly higher from at least one other co-cultivation treatment (Table 1).

Table 1: Summary of Tukey's HSD post-test for the significant pairwise differences between final yields across the nine treatments (8 co-cultivation, 1 control).

Treatments	Mean difference	Lower 95% confidence interval	Upper 95% confidence interval	p-value
<i>S. silvestris</i> with <i>T. obliquus</i> – <i>B. toyonensis</i> with <i>T. obliquus</i>	0.080	0.158	0.002	0.043
<i>S. silvestris</i> with <i>T. obliquus</i> – <i>P. fildesensis</i> with <i>T. obliquus</i>	0.098	0.177	0.020	0.008
<i>S. silvestris</i> with <i>T. obliquus</i> – <i>T. obliquus</i> control	0.131	0.209	0.053	<0.001
<i>S. xenophagum</i> with <i>T. obliquus</i> – <i>T. obliquus</i> control	0.107	0.185	0.029	0.003

<i>P. laurentiana</i> with <i>T. obliquus</i> – <i>P. fildesensis</i> with <i>T. obliquus</i>	0.085	0.163	0.007	0.026
<i>P. laurentiana</i> with <i>T. obliquus</i> – <i>T. obliquus</i> control	0.119	0.197	0.041	0.001

The eight isolated bacterial representatives were compared with the ASVs identified within the microalgal microbiome samples (grown on fully concentrated digestate, diluted digestate, or the control/f/2 medium) using the BLAST ‘align two or more sequences’ function. *M. algeriense* and *M. oxydans* were both significantly aligned with the ‘*Microbacteriaceae* sp.’ ASV, both having a 97.22% identity with this ASV. ‘*Microbacteriaceae* sp.’ had moderate relative abundance after day 0 within all treatments (Figure 4). A full table of relative abundance values for each ASV within each treatment replicate can be found in the supplementary material (Table S3). *B. aurantiaca* was significantly aligned to the ‘*Brevundimonas* sp.’ ASV, having a 100% identity with this ASV. While ‘*Brevundimonas* sp.’ had the highest relative abundance of all the ASVs, it was not evenly abundant across the samples. It was highly abundant within the day 0 samples yet declined in abundance over the following two sampling days, particularly within those samples containing digestate. *S. silvestris* was significantly aligned to the ‘*Solibacillus* sp.’ ASV, having a 100% identity with this ASV. ‘*Solibacillus* sp.’ was very abundant in the day 0 control treatment and day 0 treatment 1. However, its abundance quickly declined, and it was not present at all within the day 7 control treatment or day 7 treatment 1, with it only being present in one replicate of the day 7 treatment 2 samples in low abundance. *P. fildesensis* was significantly aligned to the ‘*Pseudomonas_E_647464* sp.’ ASV, having a 100% identity with this ASV. ‘*Pseudomonas_E_647464* sp.’ was only present in one replicate of treatment 1 collected on day 0 and one replicate of treatment 2 collected on day 7 in very low abundances. *S. xenophagum* was significantly aligned to the ‘*Sphingobium_A_485959 xenophagum*’ ASV, having a 100% identity with this ASV. ‘*Sphingobium_A_485959 xenophagum*’ was in very low abundance in the majority of samples with the exception of one replicate of the day 4 control treatment and one replicate of the day 7 control treatment. *P. laurentiana* was significantly aligned with the ‘*Pseudomonadaceae* sp.’ having a 100% identity with this ASV. ‘*Pseudomonadaceae* sp.’ was not found in any of the day 0 samples but was found in the day 4 and day 7 treatment 1 and treatment 2 samples in relatively high abundances. Finally, *B. toyonensis* was not significantly aligned with any of the ASVs present in the microalgal microbiome samples, with no matches above 97% identity.

Discussion

Traditional wastewater treatment systems are generally inefficient and unsustainable. Microalgal wastewater treatment systems may provide a viable and sustainable solution that contributes to a circular economy. Currently, there are challenges with upscaling these systems. Identifying bacterial strains within the microalgal microbiome and investigating the effects of specific strains can be the first steps when aiming to utilise microalgae-bacteria consortia to upscale and improve microalgal wastewater treatment systems. Studies investigating the bacterial communities within microalgal microbiomes that have been cultivated using wastewater, specifically digestate, are still limited. This study assessed the bacterial communities associated with the green freshwater microalgae *T. obliquus* when it was cultivated using treatments with varying concentrations of agricultural digestate, identified bacterial strains associated with *T. obliquus* during cultivation on agricultural digestate, and investigated how selected bacterial strains impact *T. obliquus* growth.

Bacterial diversity within the microalgal microbiomes

The varying levels of digestate significantly altered the bacterial diversity and evenness. This may be due to the differing levels of nitrogen and/or phosphorus concentrations found in the f/2 medium when compared with the mixture of the f/2 media and digestate and the fully concentrated digestate. While the bacteria already present within the digestate before microalgal cultivation may have impacted the communities, after 4-7 days the communities should have developed to those suited the microalgal phycosphere and may be able to form symbiotic relationships with *T. obliquus*. Therefore, the heightened ammonia and phosphate levels were likely the cause of the disparity in bacterial diversity and evenness between treatments.

Other than the diluted digestate on day 4, the bacterial diversity and evenness of the treatments containing digestate were lower than the f/2 medium across the three sampling days. On day 4, the diluted digestate had higher bacterial diversity and evenness when compared to both the f/2 medium and the fully concentrated digestate. Typically, high levels of ammonia can inhibit some species and reduce the species diversity within bacterial communities (99). However, Ruiz-Sánchez *et al.*, (100) found that, within digesters, moderate ammonia concentrations (6-7 mg/L), similar to the ammonia concentration found in the diluted digestate collected on day 4 (8 mg/L), increased the bacterial species richness when compared to lower ammonia concentrations (1-2 mg/L), similar to the concentrations found in the f/2 medium collected on day 4 (<1 mg/L). The diluted ammonia concentrations within the diluted digestate may have been at a more tolerable level for bacterial growth compared to the fully concentrated digestate and, due to the increased ammonia concentrations compared to the f/2 medium, at a level that promotes bacterial growth when compared to f/2 medium. Thus, increasing the bacterial diversity and evenness within the diluted digestate on day 4. Besides this, the bacterial diversity and evenness of the bacterial communities within the diluted digestate and fully concentrated digestate were lower than the bacterial diversity and evenness within the f/2 medium.

Microalgal species, including *T. obliquus*, have been frequently cultivated using f/2 media with success, particularly at a laboratory scale (101). The *T. obliquus* microbiome is well suited to the nitrogenous and phosphorus compound concentrations within this medium and may be less suited or require time to adapt to those differing nutrient concentrations within digestate (101). The differences in nutrient concentrations and composition between the fully concentrated and diluted digestate and the f/2 medium, as indicated in the environmental data collected previously (Table S4), may explain the lower bacterial Shannon diversity and Pielou's evenness within those treatments containing digestate. These results suggest that fewer bacterial species are able to thrive or adapt quickly to the heightened ammonia and phosphate levels that are typically found within digestate. This is consistent with previous studies, such as Li *et al.*, (102) which found the diversity of activated sludge-derived microbial communities significantly decreased after being exposed to a 10% increase in wastewater concentration (103).

However, there appeared to be no significant differences in the species richness across all three treatments, including the f/2 medium. This suggests that the significant differences between the treatments' bacterial diversity are more likely due to the evenness incorporated in the Shannon diversity equation rather than the richness. Hence, the significant difference between the treatments' bacterial diversity was likely related to the abundances of microbes, implying that within those treatments containing digestate, there were a few dominant species in large abundances, whereas the f/2 medium contained bacterial species with more even abundances which is consistent with previous literature (104). Several studies have shown that bacterial communities significantly shift when in the presence of increasing concentrations of wastewater or nutrients, such as

nitrogenous or phosphorus compounds, with some strains benefiting from the change in nutrient concentration, subsequently dominating the community and increasing in abundance (103–107). This is evident within the fully concentrated digestate as there were lower levels of bacterial evenness within these samples collected across all three sampling days when compared to the f/2 medium. Within the fully concentrated digestate, there may have been a few bacterial strains that were better suited or adapted quicker to the higher concentrations of ammonia and phosphate. These more tolerant bacterial species may have outcompeted others, therefore dominating the environment and leading to the lower levels of evenness witnessed within the fully concentrated digestate.

Bacterial community structural diversity between the microalgal microbiomes

The bacterial community compositions were significantly different depending on if the bacterial communities were cultivated using the f/2 medium, the diluted digestate or the fully concentrated digestate and changed over time. After day 0, the bacterial community compositions within the diluted digestate and fully concentrated digestate were more alike and dissimilar to the bacterial communities within the f/2 medium. Bacterial communities have been shown to be highly sensitive to changes in nutrient concentration, including ammonia concentrations, and the composition and functionality of these communities often shift when environmental changes occur (108–111). Ruprecht *et al.*, (109) found that wastewater effluents, from the run-off of traditional wastewater treatment systems, above moderate concentrations (>10%) caused significant changes in microbial community compositions and functions within river environments, with the genetic changes and responses linked to global nitrogen cycling pathways (denitrification and nitrogen fixation). This suggests that in environments with higher concentrations of nitrogenous compounds microbial communities shift to those species that can cope with and 'recycle' the nitrogenous compounds. Therefore, the heightened ammonia concentrations found within the diluted and fully concentrated digestate by previous researchers may explain the disparity witnessed in the present study between the bacterial communities within the fully concentrated or diluted digestate and the bacterial communities within the f/2 medium.

While, after day 0, the bacterial communities within the fully concentrated or diluted digestate were more alike and dissimilar to those communities within the f/2 medium, there were still differences between the bacterial communities within the diluted digestate and the fully concentrated digestate. The diluted digestate, a mixture of f/2 media and digestate, logically had diluted levels of ammonia and phosphate. Some species may be able to tolerate a diluted level of digestate, due to these diluted nutrient concentrations, yet are unable to thrive in fully concentrated digestate, thus explaining the differences witnessed in the bacterial community structures of the fully concentrated and diluted digestate. Several studies have reviewed how wastewater effluent at increasing gradients impacts bacterial communities and indicate that the compositions of bacterial communities can significantly shift at increasing wastewater concentrations and gradients (112, 113). Li *et al.*, (113) found that bacterial community compositions were significantly different between treatments with varying nitrogen gradients (within swine wastewater), suggesting that varying nitrogenous compound concentrations could influence and change bacterial communities. This study also found that bacterial abundance was reduced at higher nitrogen concentrations when compared to medium nitrogen concentrations, suggesting that bacterial species may not be able to thrive and increase in abundance at high nitrogenous compound concentrations (113). This indicates that weaker nitrogenous compound concentrations are more tolerable to a wider range of bacterial species, and these reduced concentrations allow for a greater majority of species to increase in abundance more quickly when compared to higher nitrogen concentrations (113). This may explain the differences

between the bacterial communities found within the fully concentrated digestate and the diluted digestate. Overall, the differences between these two environments are likely due to the different digestate concentrations, specifically the ammonia concentrations within the digestate, with the fully concentrated digestate being less tolerable to some bacterial species. This therefore shifts the composition of the bacterial community within the fully concentrated digestate when compared to the bacterial community found in the diluted digestate. By analysing the specific strains that were abundant within the bacterial community found in the full concentrated digestate, potential candidates for the upscaling of microalgal wastewater treatment systems may be identified.

Abundance of taxonomic classes and bacterial strains within the microalgal microbiomes

Alphaproteobacteria, *Gammaproteobacteria*, and *Bacteroidia* were the three most abundant taxonomic classes found within the microalgal microbiome samples, representing a total of 82% of the bacterial community. Both *Alphaproteobacteria* and *Gammaproteobacteria* belong to the *Proteobacteria* phylum and *Bacteroidia* belongs to the *Bacteroidota* phylum (114, 115). *Proteobacteria* and *Bacteroidota* are the most common phyla to form symbiotic relationships or be associated with microalgae (60–62). Moreover, previous studies have found these phyla to be prominent within the *T. obliquus* microbiome and many conventional wastewater treatment processes (60, 61, 64, 116). Wang *et al.*, (64) found *Gammaproteobacteria* to be the most dominant taxonomic class, with *Alphaproteobacteria* the second most abundant when isolating bacteria from *T. obliquus* (referred to as *Scenedesmus obliquus*) showing that *Proteobacteria* was overall the most prominent phyla detected. Bucheli *et al.*, (60) also found that *Proteobacteria* was the most dominant phylum when isolating bacteria from *T. obliquus* cultivated using various cultivation systems, with *Bacteroidota* being the second most abundant phylum. Most bacteria belonging to these phyla are capable of rapidly degrading organic compounds (60, 117). This allows for the direct transferral of organic carbon that microalgae provide within the phycosphere and the degradation of organic nitrogen into more usable inorganic forms for microalgae to utilise for growth (44, 60, 117, 118). This suggests that bacteria belonging to these phyla may be able to form mutualistic symbiotic relationships with *T. obliquus* primarily through the exchange of organic nutrients which may explain the dominance of these taxonomic classes within the microalgal microbiomes investigated here.

To make microalgal wastewater treatment systems most effective, the bacteria selected for microalgae-bacteria consortia need to be compatible with the microalgae as well as tolerant to high nutrient environments that are often found within wastewater (119). Further analysis of the amplicon data revealed eight ASVs of interest due to their greater abundance in the diluted digestate and/or the fully concentrated digestate on day 4 and/or day 7 when compared to the day 0 samples and the samples cultivated using f/2 medium. Four of these ASVs, all belonging to the *Proteobacteria* phylum, (*Comamonas_F_589250* sp., *Pseudomonadaceae* sp., *Burkholderiaceae_A_592522* sp., and *Sphingopyxis* sp.) were present in higher abundances within both the diluted and fully concentrated digestate on both days 4 and 7 when compared to day 0 samples and the f/2 medium.

Comamonas species, *Pseudomonadaceae* species, *Burkholderiaceae* species, and *Sphingopyxis* species have all been isolated from wastewater sources in previous studies and are often linked to denitrification processes (120–124). In the present study, *Comamonas_F_589250* sp., and *Pseudomonadaceae* sp., were the most abundant strains out of the four ASVs present in both the diluted and fully concentrated digestate on both days 4 and 7 (*Comamonas_F_589250* sp., *Pseudomonadaceae* sp., *Burkholderiaceae_A_592522* sp., and *Sphingopyxis* sp.). Ayre *et al.*, (116) found that both *Pseudomonas* (belonging to the *Pseudomonadaceae* family) and *Comamonas* genera dominated the bacterial communities within undiluted digestate. These genera have the ability to grow rapidly within nutrient-rich environments and often outcompete other microbes (116, 125).

Their ability to outcompete other microbes may be linked to the fact that species within both genera have been identified as de-nitrifying bacteria that often increase within high ammonia environments (123, 124, 126, 127). This may explain their greater abundance within the fully concentrated and diluted digestate which was found to contain a higher concentration of ammonia when compared to the f/2 medium (127). As *Pseudomonadaceae* and *Comamonas* species are well suited to high ammonia environments, they may be able to metabolise or utilise this nitrogenous compound more efficiently than other bacterial species. In fact, Wang *et al.*, (124) found that *Comamonas* species were involved in alleviating the ammonia toxicity for microalgal species within high ammonia concentrations. This suggests that *Comamonas* species may contribute to microalgal growth by reducing the limiting ammonia concentrations and creating a more tolerable environment. Furthermore, *Pseudomonas* species are known to form symbiotic relationships with microalgae, such as *Chlorella* species, often increasing *Chlorella* growth and the nitrogen elimination within wastewater (128, 129). These characteristics are likely the reason *Comamonas*_F_589250 sp., and *Pseudomonadaceae* sp., were found in large abundance within the diluted and fully concentrated digestate, as these contained higher ammonia concentrations, and make these ASVs good candidates for microalgal wastewater treatment systems due to the relationships they have been observed to form with microalgae.

The other four ASVs of interest (SIO2C1 sp010672925, *Runella zae*, *Pigmentiphaga* sp., and *Reyranella aquatilis*) were only present in greater abundance in one of the two treatments containing digestate on days 4 and 7 or in both treatments containing digestate on either day 4 or 7 when compared to the samples cultivated using f/2 medium and day 0 samples. *Runella* and *Pigmentiphaga* species have both been isolated from wastewater previously, and *Reyranella aquatilis* has been found to thrive in eutrophic lakes (130–134). These findings suggest that *Runella zae*, *Pigmentiphaga* sp., and *Reyranella aquatilis* may be able to tolerate wastewater and nutrient-rich environments so may be suitable isolates for microalgal wastewater treatment. However, these isolates were not present in both the diluted digestate and the fully concentrated digestate on both days 4 and 7, and when compared to *Comamonas*_F_589250 sp., and *Pseudomonadaceae* sp., they were in remarkably lower abundances. This suggests that they may not be as tolerant and flexible to the nutrient-rich environments typically found within digestate and therefore as useful as *Comamonas*_F_589250 sp., *Pseudomonadaceae* sp., *Burkholderiaceae*_A_592522 sp., and *Sphingopyxis* sp. Additionally, due to their low abundances, they may not be critical for *T. obliquus* growth and consequently may not improve the efficiency of *T. obliquus* wastewater treatment systems. While some low abundance bacterial species may still be ecologically important, for the purpose of their use within microalgal wastewater treatment systems they may not be as suited to the less tolerable environment and therefore not as critical for *T. obliquus* growth within these environments. Ultimately, the main isolates of interest are *Comamonas*_F_589250 sp., *Pseudomonadaceae* sp., *Burkholderiaceae*_A_592522 sp., and *Sphingopyxis* sp. due to their presence in both treatments containing digestate on both days 4 and 7, with *Comamonas*_F_589250 sp., and *Pseudomonadaceae* sp., being of particular interest due to their large relative abundance. These strains should be investigated further to determine whether they may be viable candidates for larger-scale *T. obliquus* wastewater treatment systems.

Functionality of the bacterial communities within the microalgal microbiomes

The bacterial communities within the microalgal microbiomes were predominantly associated with metabolism functions according to Tax4Fun2 functionality analysis. This is to be expected as mutualistic symbiotic relationships between microalgae and bacteria often involve the exchanging of nutrients (42–44). Therefore, the bacterial functions within these communities are predicted to be

associated with metabolising carbohydrates, proteins, and other nutrients that are beneficial for microalgal growth. Overall, the majority of samples had uniform abundances of functional pathways.

Global and overview maps, amino acid metabolism, carbohydrate metabolism, membrane transport, and signal transduction were some of the functional pathways with relatively high abundance within the microalgal microbiomes. Within global and overview maps specifically, metabolic pathways, microbial metabolism in diverse environments, and biosynthesis of secondary metabolites were the most abundant functional traits. This further shows that within the microalgal microbiome samples, the primary functional characteristics were associated with metabolism and interestingly metabolism within diverse environments. This may suggest that some of the bacterial strains within the samples are well suited to metabolising nutrients within varying environments such as digestate or nutrient-rich environments. Furthermore, the primary functional pathway within membrane transport was identified as ABC transporters which transport substrates such as ions, sugars, lipids, and proteins (135). These could all be transported to provide for microalgal growth or transported from microalgal sources for bacterial use. Finally, signal transduction was also an abundant functional pathway. Signal transduction refers to the detection of chemical or physical signals at the cell surface (136). Bacteria respond to the enriched nutrient gradients found close to microalgae cells and within the phycosphere (137–139). This signal transduction pathway could be linked to this response which allows the bacteria to benefit from the nutrients within the phycosphere and possibly provide microalgae with growth promoting substrates and other nutrients (137–139). Overall, the functional pathways identified through Tax4Fun2 analysis indicate that the bacteria within the *T. obliquus* microbiomes are well suited for interactions and mutualistic symbiotic relationships with microalgae, as expected. As the majority of samples had uniform abundances of functional pathways, there appear to be no major changes in the functional profile of bacterial communities based on digestate concentration.

Co-cultivation of *T. obliquus* and bacterial isolates

To determine the impact that certain bacteria had on *T. obliquus* growth, separate co-cultivations of eight bacterial isolates and *T. obliquus* were conducted. Overall, there were four bacterial strains that, when co-cultivated with *T. obliquus*, had higher growth rates than the *T. obliquus* control (*S. silvestris*, *B. aurantiaca*, *M. oxydans*, and *M. algeriense*) and three bacterial strains that had significantly different final yields when compared to the *T. obliquus* control (*S. silvestris*, *S. xenophagum*, and *P. laurentiana*).

The bacterial strains that increased the *T. obliquus* growth rate likely have a symbiotic relationship with the microalgae and can help to stimulate and increase growth at a faster rate when compared with axenic cultures of *T. obliquus*. Two of the strains that significantly increased the growth rate, when compared to the *T. obliquus* monoculture, belonged to the *Microbacterium* genus. This genus has been reported to exhibit useful characteristics associated with environmental biotechnology (140). *Microbacterium* species are often nitrogen-fixing bacteria and have been identified to promote growth when cultured with both plant and microalgal species (140–143). Bacterial species within this genus have been identified within the microbiomes of microalgal species belonging to the *Chlorella* genus (144, 145). Studies reviewing how bacterial species impact *Chlorella* species growth have concluded that *Microbacterium* species often promote growth and that microbes belonging to these two genera often form symbiotic relationships (144, 145). This suggests that the two *Microbacterium* species co-cultured with *T. obliquus* here may form symbiotic relationships with *T. obliquus* and provide the microalgae with nitrogen to promote faster growth rates.

As with the present study, *Brevundimonas* species, such as *B. aurantiaca*, have been found within microalgal microbiomes, including *T. obliquus*, in the past and have been found to promote the growth of *Chlorella* and *Tetrademus* species (60, 64, 146). Park *et al.*, (146) found that a bacterial species most likely belonging to the *Brevundimonas* genus increased the growth of *Chlorella ellipsoidea* and witnessed the direct adhesion of the bacterial cells to the surface of the microalgal cells. This study also observed the development of crinkles on the surface of the microalgae within the co-cultures containing *Brevundimonas* species (146). Direct adhesion of the microalgal and bacterial cells may lead to a more rapid exchange of useful nutrients due to the diminished diffusion distance between the cells and the crinkled cell surface observed on the microalgal cells may further indicate that there is an exchange of useful nutrients between the microalgae and bacteria (146). Overall, this suggests that *Brevundimonas* species can form positive symbiotic relationships with microalgae which results in increased microalgal growth and may explain the increased growth rate that *B. aurantiaca* induced within *T. obliquus* co-cultures. To identify if the isolate *B. aurantiaca* within this study forms similar adhesions with *T. obliquus* which may explain the rapid growth rates, scanning electron microscopy must be conducted to observe the interactions between the two microbes.

While these three bacterial isolates (*B. aurantiaca*, *M. oxydans*, and *M. algeriense*) significantly increased the growth rate of *T. obliquus* when compared to the axenic culture of *T. obliquus*, they did not produce significantly different final yields to the axenic *T. obliquus* culture. Only the co-cultivation treatment containing *S. silvestris* had a significantly greater growth rate and final yield when compared with the axenic culture of *T. obliquus*. At the time of writing, *S. silvestris* has not been identified to increase the growth rate or increase the yield of *T. obliquus* or another microalgal species previously. However, it can produce a cost-effective biofloculant for microalgal harvest (147–149). This is of interest as, in this study, *S. silvestris* has been shown to increase *T. obliquus* growth which may make microalgal wastewater treatment systems more efficient and sustainable, and in previous studies, it has been shown to produce a biofloculant that makes the harvesting of microalgae more efficient (147). These characteristics suggest that *S. silvestris* may be an ideal candidate for aiding the upscaling of microalgal wastewater systems. However, analysis of the microalgal microbiomes using the amplicon data from the first part of the study showed that *S. silvestris* was present in higher abundances within the day 0 samples when compared to the day 4 and 7 samples. This suggests that it may have been outcompeted by other bacterial strains that were better suited to the nutrient-rich digestate within the samples. Therefore, it may not be as suitable as presented in the co-cultivation study for microalgal wastewater treatment of nutrient-rich wastewater or digestate. Alongside the co-cultivation treatment containing *S. silvestris*, the treatments containing *S. xenophagum*, and *P. laurentiana* also had significantly different final yields when compared to the axenic culture of *T. obliquus*.

Unlike *Sphingobium* species, including *S. xenophagum*, species belonging to the *Pseudomonas* genus, such as *P. laurentiana*, have been identified previously to induce or promote microalgal growth (64, 150, 151). Mouget *et al.*, (150) found that *Pseudomonas diminuta* and *Pseudomonas vesicularis* could promote the growth of both *Tetrademus* (referred to as *Scenedesmus*) and *Chlorella* microalgal species. Moreover, Mujtaba *et al.*, (129) found that a co-culture of *Pseudomonas putida* and *Chlorella vulgaris* increased the removal of nutrients when compared to the axenic cultures of both species. *Pseudomonas* species are denitrifying bacteria and are therefore useful within microalgal wastewater treatment systems to recycle and break down the high nitrate and ammonia concentrations often found within digestate and agricultural wastewater (123, 152). As *P. laurentiana* significantly increased the final yield of *T. obliquus*, and *Pseudomonas* species are denitrifying bacteria, *Pseudomonas* species, and possibly *P. laurentiana* may be suitable and effective

candidates for microalgal wastewater treatment utilising *T. obliquus*. When analysing the microalgal microbiomes using the amplicon data from the first part of this study, the ASV identified as '*Pseudomonadaceae* sp.' was significantly aligned with *P. laurentiana* and was very abundant within the samples containing digestate. This further implies that the isolate *P. laurentiana* may be a useful and suitable candidate for *T. obliquus* wastewater treatment and requires further analysis within larger scale microalgal wastewater treatment systems.

Limitations and further work

Although this study has identified potential strains for microalgal wastewater treatment systems, these conclusions were made through two separate experiments. Ideally co-cultivation experiments would have been conducted within digestate or wastewater to be able to determine which strains are able to promote microalgal growth while within a less tolerable environment. Due to time constraints and availability of materials this was not possible. In future co-cultivation studies with multiple bacterial strains should be conducted to analyse the impacts of competition on microalgal growth. Finally, larger scale co-cultivation and cultivation on digestate experiments should be carried out to determine if the bacterial strains identified in this study are viable for microalgal wastewater treatment systems at large scales.

Conclusion

Overall, this study showed that the microalgal microbiomes cultivated using digestate had significantly different bacterial community compositions with lower bacterial Shannon diversity and Pielou's evenness when compared to those microbiomes cultivated on traditional f/2 media. This suggests that digestate can dictate and change *T. obliquus* bacterial communities likely due to the enriched nutrient concentrations within this environment. Therefore, when designing microalgal and *T. obliquus* wastewater treatment systems that utilise bacteria it is important to consider and test which bacteria are well suited to the wastewater environments and can thrive in these, often, less tolerable conditions. *Proteobacteria* and *Bacteroidota* were the most abundant phyla found across the microalgal microbiomes. These are the most common phyla to form symbiotic relationships with microalgae (60). Two ASVs, namely *Comamonas*_F_589250 sp. and *Pseudomonadaceae* sp., belonging to the *Proteobacteria* phylum were of interest due to their abundance both overall and particularly within treatments containing digestate. *Comamonas*_F_589250 sp. and *Pseudomonadaceae* sp. were found in relatively high abundance within both treatments containing digestate on both days 4 and 7. These isolates may make good candidates for microalgal wastewater treatment systems using *T. obliquus* due to their abundance within and tolerance to digestate. Co-cultivation of *T. obliquus* and individual bacterial strains revealed that *P. laurentiana*, belonging to the *Pseudomonadaceae* family and significantly aligned with the '*Pseudomonadaceae* sp.' ASV, significantly increased the final yield of *T. obliquus* when compared to the axenic culture of *T. obliquus*. This indicates that the *P. laurentiana* isolate may be a good candidate for microalgal wastewater treatment systems due to its positive impact on *T. obliquus* growth and ability to tolerate and thrive within digestate and nutrient-enriched environments. This isolate and bacterial species within the *Pseudomonadaceae* family should be investigated when aiming to upscale *T. obliquus* and microalgal wastewater treatment systems.

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Appendices

Figure S1 – Bioinformatic workflow

(https://otagoedna.github.io/getting_started_with_qiime2/overview.html)

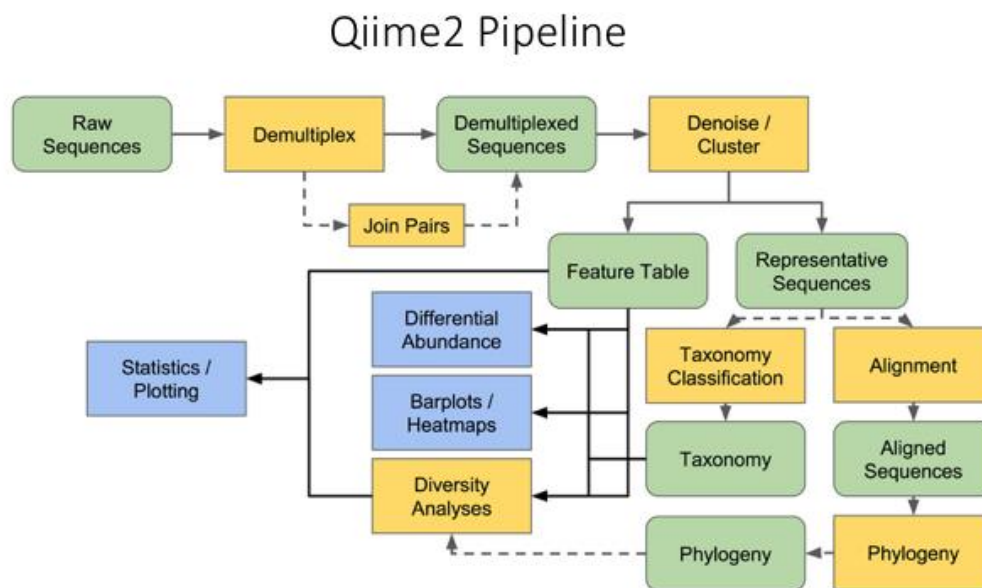


Figure S2 – Co-cultivation 96-well plate set up

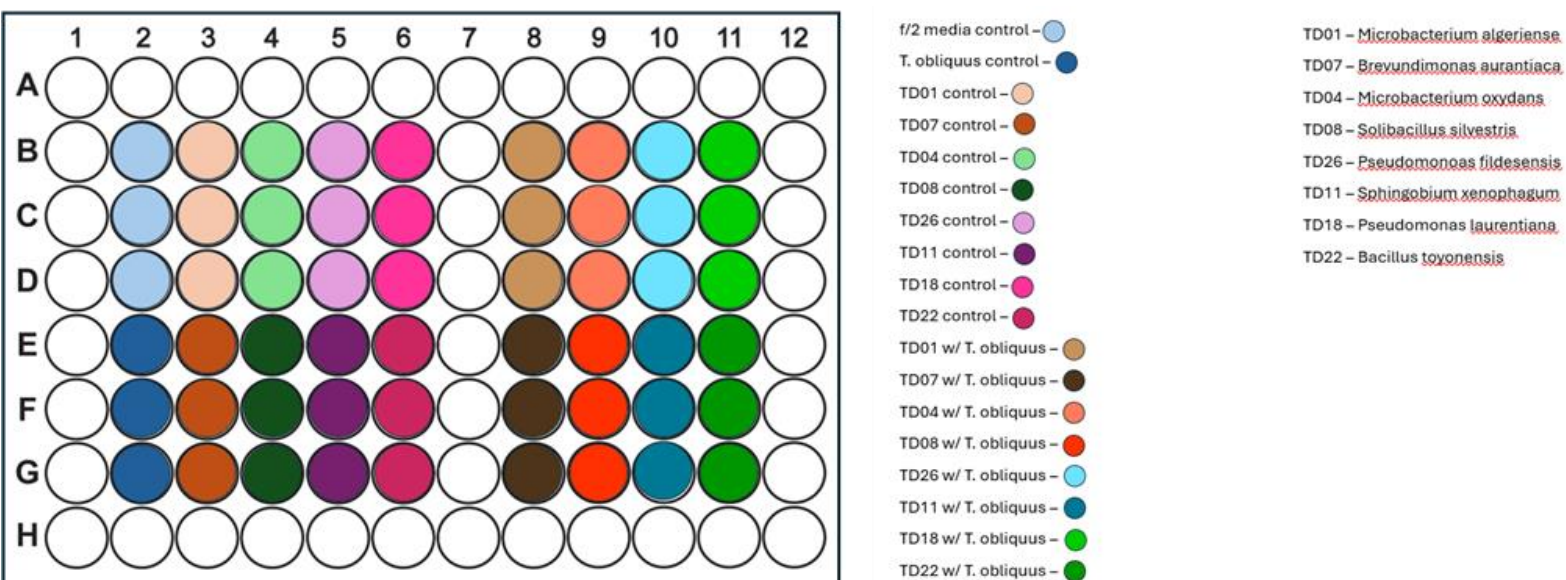


Table S1 – Kruskal Wallis test to determine differences between level 2 KEGG pathway abundances between samples. Chi-squared (χ^2), degrees of freedom (df), and p-value from the Kruskal-Wallis test presented, * indicating a significant p-value ($p < 0.05$). Dunn-Bonferroni post-hoc test results presented showing the specific samples that had differing abundances of Level 2 functional pathways.

Level 2 Functional pathways	χ^2	df	p-value	Dunn-Bonferroni post-hoc test significant difference results
Aging	15.598	8	0.049*	Between day 0, control and day 7, treatment 2 ($p = 0.037$).
Amino acid metabolism	19.344	8	0.013*	Between day 0, control and day 7, treatment 2 ($p = 0.031$).
Biosynthesis of other secondary metabolites	18.559	8	0.017*	No significant differences.
Cancers: Overview	19.894	8	0.011*	No significant differences.
Cancers: Specific types	18.554	8	0.017*	No significant differences.
Carbohydrate metabolism	19.651	8	0.011*	No significant differences.
Cardiovascular diseases	16.922	8	0.031*	No significant differences.
Cell growth and death	16.656	8	0.034*	No significant differences.
Cell motility	19.831	8	0.011*	No significant differences.
Cellular community - prokaryotes	20.201	8	0.010*	Between day 0, control and day 7, treatment 1 ($p = 0.022$).
Chemical structure transformation maps	17.598	8	0.024*	No significant differences.
Circulatory system	22.148	8	$<0.001^*$	Between day 0, control and day 7, treatment 1 ($p < 0.01$).
Development	20.252	8	0.009*	Between day 0, control and day 7, treatment 1 ($p = 0.043$).
Digestive system	23.478	8	0.003*	Between day 4, treatment 2 and day 7, control ($p = 0.026$). Between day 7, control and day 7, treatment 2 ($p = 0.015$).
Drug resistance: Antimicrobial	15.069	8	0.058	Between day 0, treatment 2 and day 4, control ($p = 0.012$).
Drug resistance: Antineoplastic	16.265	8	0.039*	Between day 0, control and day 7, treatment 2 ($p = 0.012$).
Endocrine and metabolic diseases	19.386	8	0.013*	Between day 4, control and day 7, treatment 2 ($p = 0.037$). Between day 7, treatment 1 and day 7, treatment 2 ($p = 0.026$).
Endocrine system	19.175	8	0.014*	Between day 0, control and day 7, treatment 1 ($p = 0.026$).
Energy metabolism	16.307	8	0.038*	No significant differences.
Environmental adaptation	16.138	8	0.040*	Between day 7, control and day 7, treatment 1 ($p = 0.018$).
Excretory system	10.972	8	0.203	No significant differences.
Folding, sorting and degradation	14.851	8	0.062	No significant differences.
Global and overview maps	17.545	8	0.025*	Between day 0, control and day 4, control ($p = 0.037$).
Glycan biosynthesis and metabolism	16.656	8	0.034*	Between day 4, treatment 2 and day 7, treatment 1 ($p < 0.01$).
Immune diseases	16.434	8	0.037*	Between day 7, control and day 7, treatment 1 ($p = 0.031$).
Immune system	18.265	8	0.019*	Between day 4, control and day 4, treatment 2 ($p = 0.043$). Between day 4, treatment 2 and day 7, treatment 1 ($p = 0.021$).
Infectious diseases: Bacterial	12.667	8	0.124	No significant differences.
Infectious diseases: Parasitic	18.847	8	0.016*	Between day 7, treatment 1 and day 7, treatment 2 ($p = 0.037$).
Infectious diseases: Viral	23.984	8	0.002*	Between day 4, treatment 2 and day 7, treatment 1 ($p = 0.031$). Between day 7, treatment 1 and day 7, treatment 2 ($p = 0.018$).
Lipid metabolism	20.646	8	0.008*	Between day 0, control and day 7, treatment 1 ($p < 0.01$).
Membrane transport	15.672	8	0.047*	Between day 0, control and day 7, treatment 1 ($p = 0.026$).
Metabolism of cofactors and vitamins	17.714	8	0.023*	No significant differences.
Metabolism of other amino acids	21.037	8	0.007*	No significant differences.
Metabolism of terpenoids and polyketides	23.093	8	0.003*	Between day 0, control and day 4, treatment 2 ($p = 0.023$). Between day 0, control and day 7, treatment 2 ($p = 0.015$). Between day 7, control and day 7, treatment 2 ($p = 0.033$).
Nervous system	10.704	8	0.219	No significant differences.
Neurodegenerative diseases	17.185	8	0.028*	Between day 4, control and day 7, treatment 2 ($p = 0.033$).
Nucleotide metabolism	21.175	8	0.007*	Between day 0, treatment 1 and day 4, treatment 2 ($p = 0.037$). Between day 0, treatment 1 and day 7, treatment 2 ($p < 0.01$).
Replication and repair	15.245	8	0.055	Between day 0, treatment 1 and day 4, treatment 2 ($p = 0.038$).
Signal transduction	5.894	8	0.659	No significant differences.
Signaling molecules and interaction	16.725	8	0.033*	No significant differences.

Substance dependence	17.016	8	0.030*	Between day 0, control and day 7, treatment 1 ($p = 0.015$).
Transcription	19.581	8	0.012*	No significant differences.
Translation	17.979	8	0.021*	Between day 0, treatment 1 and day 4, treatment 2 ($p = 0.022$).
				Between day 0, treatment 1 and day 7, treatment 2 ($p = 0.037$).
Transport and catabolism	23.228	8	0.003*	Between day 0, control and day 7, treatment 1 ($p < 0.01$).
Xenobiotics biodegradation and metabolism	16.804	8	0.032*	No significant differences.

Table S2 – Full table showing the t statistic and Benjamini-Hochberg adjusted p-value for each ASV within each sample from ALDEx2 analysis. * indicates a significant p value <0.05.

ASV / Taxon	Day 0, control: t. value	Day 0, treatment 1: t. value	Day 0, treatment 2: t. value	Day 4, control: t. value	Day 4, treatment 1: t. value	Day 4, treatment 2: t. value	Day 7, control: t. value	Day 7, treatment 1: t. value	Day 7, treatment 2: t. value	Day 0, control: p-value	Day 0, treatment 1: p-value	Day 0, treatment 2: p-value	Day 4, control: p-value	Day 4, treatment 1: p-value	Day 4, treatment 2: p-value	Day 7, control: p-value	Day 7, treatment 1: p-value	Day 7, treatment 2: p-value
Microbacteriaceae sp.	0.687	1.417	0.740	3.676	3.660	1.949	4.010	3.394	3.327	0.797	0.936	0.989	0.022*	0.016*	0.310	0.011*	0.022*	0.023*
<i>Mycobacterium</i> sp.	0.590	-2.705	-2.315	-2.408	-2.531	-2.601	-2.408	-2.616	-2.599	0.919	0.500	0.620	0.339	0.203	0.180	0.301	0.189	0.141
<i>Rhodococcus_C_375578</i> sp.	-3.352	-0.126	2.367	-0.059	-0.225	3.567	-0.023	-0.123	3.391	0.099	0.968	0.526	0.915	0.873	0.063	0.912	0.855	0.068
<i>Nanopelagicus</i> sp.	-1.327	0.047	-0.708	-0.913	-1.058	-1.191	-0.953	-1.053	-1.155	0.579	0.995	0.968	0.839	0.701	0.618	0.798	0.676	0.612
SYFI01 sp009694365	-0.998	-1.456	-1.186	-1.323	-1.348	-1.296	-1.219	-1.301	-1.393	0.702	0.830	0.903	0.693	0.572	0.600	0.698	0.603	0.513
<i>Paludibacter</i> sp001873635	-2.997	1.236	0.330	-0.021	-0.014	-0.113	-0.036	-0.075	-0.246	0.119	0.839	0.962	0.942	0.923	0.885	0.922	0.912	0.861
<i>Tenuifilaceae</i> sp.	15.294	1.250	1.114	3.159	-2.130	-3.850	2.722	-4.736	-1.141	<0.001*	0.955	0.978	0.045*	0.214	0.016*	0.097	0.002*	0.631
Chitinophagaceae_966727 sp.	14.552	0.199	-0.417	3.638	3.173	0.701	2.937	4.925	2.047	<0.001*	0.998	0.998	0.024*	0.042*	0.962	0.076	0.002*	0.247
<i>Ferruginibacter</i> sp.	-1.024	-1.373	-1.151	-1.255	-1.388	-1.432	-1.333	-1.333	-1.486	0.705	0.861	0.892	0.728	0.568	0.513	0.679	0.602	0.488
<i>Flavihumibacter</i> sp.	3.869	1.122	0.303	3.418	2.091	2.373	5.057	4.118	4.050	0.005*	0.978	1.000	0.030*	0.274	0.166	0.002*	0.006*	0.007*
<i>Panacibacter_956521</i> sp.	7.567	0.541	-0.316	2.859	1.746	0.821	2.191	0.907	-0.526	<0.001*	0.999	1.000	0.065	0.448	0.981	0.201	0.965	0.972
<i>Dyadobacter_906144</i> sp.	15.049	0.761	-0.333	0.107	-3.649	-4.462	-0.527	-6.356	-5.436	<0.001*	0.994	1.000	1.000	0.035*	0.009*	0.997	<0.001*	<0.001*
<i>Dyadobacter jiangsuensis</i>	5.868	0.574	0.692	-1.940	-1.854	-2.809	-3.862	-8.111	-3.291	0.005*	0.997	0.996	0.459	0.356	0.116	0.047*	0.002*	0.063
<i>Flectobacillus fontis</i>	-1.022	-1.384	-1.050	-1.179	-1.391	-1.400	-1.218	-1.337	-1.476	0.701	0.862	0.952	0.766	0.579	0.530	0.692	0.582	0.479
<i>Runella zeae</i>	8.529	2.037	1.525	6.927	6.740	-0.220	6.408	7.727	1.259	<0.001*	0.641	0.875	<0.001*	<0.001*	0.995	<0.001*	<0.001*	0.725
<i>Flavobacterium</i> sp.	-1.056	-0.175	-0.976	-1.253	-1.386	-1.345	-1.220	-1.337	-1.485	0.698	0.997	0.954	0.713	0.570	0.573	0.724	0.584	0.467
<i>Flavobacterium tiangeerense</i>	-1.191	-1.305	-1.075	-1.195	-1.309	-1.339	-1.189	-1.338	-1.371	0.618	0.865	0.915	0.721	0.611	0.574	0.736	0.571	0.521
PHOS-HE28 sp902825795	-4.578	1.099	0.339	4.672	5.525	4.092	7.648	7.913	6.410	0.030*	0.815	0.908	0.018*	0.005*	0.034*	<0.001*	<0.001*	0.001*
<i>Mucilaginibacter_A limnophilus</i>	3.040	-0.319	-0.188	-1.109	-4.738	-4.889	-3.318	-4.738	-4.948	0.053	1.000	1.000	0.867	0.016*	0.011*	0.084	0.008*	0.005*
<i>Pedobacter_887417</i> sp.	-0.918	-1.354	-1.021	-1.076	-1.087	-0.447	-0.988	0.067	2.659	0.760	0.889	0.942	0.794	0.698	0.888	0.801	0.988	0.091
<i>Pedobacter chitinilyticus</i>	-3.161	-0.249	0.180	-0.076	-0.120	-0.195	0.025	0.517	3.750	0.097	0.971	0.965	0.932	0.902	0.886	0.930	0.861	0.029*
Cyanobacteriales sp.	17.966	-0.109	1.877	-1.004	-3.029	-3.317	-0.112	-5.150	-7.002	<0.001*	1.000	0.718	0.895	0.072	0.034*	0.999	0.001*	<0.001*
SIO2C1 sp010672925	22.698	-0.434	2.663	2.364	6.604	6.195	2.900	1.764	-3.413	<0.001*	1.000	0.369	0.179	<0.001*	<0.001*	0.086	0.414	0.026*
Microcystaceae_A_24131 sp.	-0.969	-0.167	-0.900	-1.058	-1.152	-1.268	-0.403	-1.226	-1.350	0.715	0.997	0.967	0.779	0.681	0.605	0.958	0.635	0.554
<i>Solibacillus</i> sp.	-2.117	6.830	6.732	-0.818	0.730	3.280	-0.690	-0.959	0.293	0.281	0.002*	0.002*	0.808	0.875	0.055	0.833	0.683	0.920
<i>Mesobacillus_A_295502</i> sp.	-3.281	3.992	4.357	-0.085	-0.358	0.568	-0.158	-0.239	-0.449	0.084	0.103	0.059	0.933	0.848	0.856	0.915	0.871	0.790
Patescibacteria sp.	-1.100	-1.359	-1.055	-1.302	-1.474	-1.431	-1.262	-1.438	-1.436	0.655	0.876	0.934	0.706	0.546	0.541	0.702	0.562	0.507
UBA11704 sp.	-2.902	1.031	0.084	-0.016	-0.120	-0.239	0.064	-0.113	-0.192	0.129	0.895	0.978	0.937	0.914	0.870	0.939	0.922	0.874
UBA6899 sp.	-1.384	-0.018	-0.670	-1.024	-1.010	-1.015	-0.875	-1.002	-1.127	0.542	0.997	0.966	0.765	0.723	0.684	0.815	0.691	0.616
<i>Paludisphaera</i> sp001610895	-3.234	2.061	3.187	-0.012	-0.152	-0.135	-0.022	-0.118	-0.405	0.089	0.626	0.237	0.930	0.925	0.888	0.924	0.906	0.799
UBA1268 sp.	-1.094	-1.211	-1.077	-1.202	-1.343	-1.445	-1.183	-1.355	-1.471	0.671	0.888	0.937	0.729	0.570	0.542	0.718	0.576	0.503
UBA4655 sp.	-1.134	-1.415	-1.054	-1.183	-1.184	-1.256	-1.215	-1.340	-1.374	0.659	0.838	0.919	0.735	0.648	0.587	0.691	0.569	0.512
Alphaproteobacteria sp.	20.102	0.593	-0.236	-3.267	-2.573	-3.234	-0.239	-3.574	-4.662	<0.001*	0.991	0.998	0.096	0.146	0.049*	0.992	0.026*	0.003*
<i>Roseomonas_A_507058</i> sp.	3.930	0.038	0.062	-1.429	-2.483	-2.315	-1.824	-1.971	-0.676	0.013*	1.000	1.000	0.696	0.156	0.162	0.426	0.269	0.932
<i>Brevundimonas</i> sp.	26.049	0.741	0.157	-0.449	-2.746	-4.135	-2.592	-5.257	-6.991	<0.001*	0.991	1.000	0.996	0.103	0.013*	0.171	0.001*	<0.001*
<i>Caulobacter_487784</i> sp.	-2.506	1.776	1.637	1.909	2.006	2.326	1.641	0.531	-0.134	0.185	0.713	0.771	0.408	0.382	0.242	0.506	0.903	0.898
<i>Phenylobacterium kunshanense</i>	4.583	0.732	-0.055	3.582	0.955	1.767	4.680	2.341	3.630	0.002*	0.993	1.000	0.024*	0.955	0.420	0.003*	0.149	0.014*
<i>Reyranella aquatilis</i>	-2.466	0.837	0.145	0.669	1.000	-0.130	1.857	4.206	3.908	0.191	0.932	0.991	0.874	0.836	0.934	0.436	0.012*	0.017*
<i>Rhizobium_A_501058</i> sp.	14.078	-0.664	-1.636	-4.338	-3.216	-3.490	-3.326	-6.802	-7.540	<0.001*	1.000	0.921	0.018*	0.058	0.027*	0.062	<0.001*	<0.001*
Rhizobiaceae_A_499470 sp.	-2.399	-0.172	-1.097	2.322	2.222	2.470	3.726	2.507	-1.575	0.211	0.977	0.847	0.238	0.297	0.207	0.034*	0.175	0.477
<i>Pseudochrobactrum</i> sp.	-3.131	-0.162	0.253	0.053	0.011	0.956	0.036	0.927	3.775	0.094	0.978	0.970	0.936	0.941	0.794	0.938	0.808	0.025*
<i>Kaistia</i> sp.	-3.686	-0.296	0.103	0.086	-0.076	-0.287	-0.027	3.674	3.172	0.066	0.938	0.956	0.895	0.887	0.813	0.915	0.046*	0.086
<i>Devosia_A_501803</i> sp.	-2.998	-0.116	-0.022	0.072	-0.159	0.598	1.049	3.308	3.108	0.117	0.987	0.978	0.937	0.896	0.865	0.730	0.059	0.073

<i>Devosia_A_502124</i> sp.	17.938	0.571	-0.544	-6.367	-5.275	-4.151	-7.334	-9.526	-5.935	<0.001*	0.991	0.999	0.001*	0.005*	0.013*	<0.001*	<0.001*	<0.001*
<i>Bosea</i> sp.	24.663	-0.668	0.575	-2.244	-2.240	-3.084	-1.146	-6.760	-9.375	<0.001*	1.000	0.996	0.319	0.195	0.048*	0.811	<0.001*	<0.001*
<i>Methylobacterium jeotgali</i>	-0.186	-0.112	-0.148	-0.165	-1.046	-1.865	0.962	-1.901	-1.996	0.964	1.000	1.000	0.994	0.719	0.364	0.891	0.356	0.299
<i>Ancylobacter_B_502330</i> sp.	26.192	-1.210	-3.319	-0.445	-3.982	-4.155	1.007	-5.943	-8.083	<0.001*	0.983	0.259	0.989	0.028*	0.014*	0.831	<0.001*	<0.001*
<i>Bradyrhizobium</i> sp.	18.004	0.620	0.626	0.265	-0.629	-3.455	-0.247	-4.068	-5.253	<0.001*	0.992	0.992	0.999	0.944	0.028*	0.998	0.008*	0.001*
<i>Rhodobacteraceae</i> sp.	-1.102	-0.822	-0.571	0.008	-0.237	-0.812	1.286	0.625	-0.322	0.686	0.987	0.987	0.991	0.965	0.789	0.690	0.936	0.914
<i>Cypionkella</i> sp.	-1.121	-1.235	-0.951	-1.183	-1.269	-1.337	-1.173	-1.304	-1.341	0.640	0.857	0.946	0.724	0.608	0.581	0.733	0.604	0.538
<i>Blastomonas</i> sp.	-2.526	1.130	0.133	0.141	0.064	-0.117	-0.019	-0.086	-0.134	0.194	0.876	0.978	0.937	0.949	0.904	0.937	0.917	0.921
<i>Novosphingobium capsulatum</i>	5.143	0.462	0.381	-1.979	-3.589	-6.874	-2.260	-6.728	-6.928	0.006*	1.000	1.000	0.444	0.053	0.003*	0.308	0.002*	0.001*
<i>Novosphingobium nitrogenifigens</i>	4.448	0.596	0.329	-3.066	0.737	1.002	-4.326	-0.239	-0.422	0.006*	1.000	1.000	0.118	0.994	0.937	0.020*	1.000	0.994
<i>Novosphingobium_485351</i> sp.	0.155	0.896	1.096	-1.994	-2.228	-1.343	-1.978	-1.343	-1.268	0.986	0.984	0.977	0.473	0.278	0.553	0.441	0.571	0.558
<i>Polymorphobacter_A_486811 multimanifer</i>	0.625	1.380	1.424	3.796	3.478	3.590	4.306	3.038	2.802	0.795	0.921	0.899	0.019*	0.022*	0.019*	0.007*	0.040*	0.056
<i>Rhizorhabdus_483366</i> sp.	13.486	1.395	0.610	3.276	0.585	-2.084	2.667	-1.558	-2.169	<0.001*	0.920	0.993	0.043*	0.987	0.215	0.116	0.455	0.173
<i>Sphingobium_A_485959</i> sp.	-3.814	-0.134	0.045	1.990	4.830	6.477	-0.063	3.925	5.172	0.072	0.962	0.955	0.435	0.016*	0.002*	0.899	0.043*	0.009*
<i>Sphingobium_A_485959 alorifonticola</i>	1.666	0.156	-0.086	-1.418	-2.690	-3.481	-2.184	-3.412	-3.558	0.388	1.000	1.000	0.702	0.145	0.046*	0.307	0.046*	0.032*
<i>Sphingobium_A_485959 xenophagum</i>	3.471	0.112	-0.291	2.221	-0.140	-0.384	1.530	-0.933	-3.026	0.017*	1.000	1.000	0.213	1.000	0.998	0.546	0.829	0.036*
<i>Sphingobium_A_485959 yanoikuyae</i>	-1.228	-0.628	-0.438	0.596	-0.146	0.417	0.249	-0.031	-0.685	0.619	0.988	0.990	0.957	0.971	0.986	0.979	0.992	0.831
<i>Sphingomonas_L_486704</i> sp.	20.712	2.078	2.037	-4.045	-2.977	-2.892	-5.093	-2.875	-4.715	<0.001*	0.628	0.648	0.028*	0.080	0.062	0.005*	0.064	0.002*
<i>Sphingopyxis</i> sp.	0.580	2.578	1.430	4.109	5.807	5.657	4.647	5.918	5.382	0.823	0.378	0.901	0.013*	0.001*	0.001*	0.004*	0.001*	0.001*
<i>Sphingopyxis terrae</i>	-5.029	-0.168	0.329	6.050	7.150	6.960	7.842	7.361	6.180	0.020*	0.938	0.887	0.005*	0.001*	0.001*	0.001*	0.001*	0.003*
<i>Burkholderiaceae_A_592522</i> sp.	-1.230	-1.466	-1.186	2.795	3.901	3.713	3.097	3.985	3.713	0.609	0.814	0.890	0.094	0.015*	0.021*	0.058	0.012*	0.017*
<i>Comamonas_F_589250</i> sp.	-1.725	-1.333	1.236	4.486	6.254	5.934	3.577	6.161	5.328	0.402	0.828	0.877	0.012*	0.001*	0.002*	0.034*	0.001*	0.003*
<i>Hylemonella_582308</i> sp.	-1.210	-0.151	-0.978	-1.042	-1.193	-1.246	-1.101	-1.151	-1.331	0.627	0.996	0.935	0.790	0.652	0.592	0.749	0.657	0.548
<i>Rhodoferax_A_585629</i> sp.	-1.044	-1.248	-0.216	-1.087	-1.140	-1.268	-1.064	-1.133	-1.299	0.704	0.885	0.991	0.771	0.674	0.585	0.748	0.674	0.548
<i>Variovorax</i> sp.	-2.917	-0.128	0.133	1.178	4.377	3.547	0.114	3.138	2.418	0.109	0.976	0.976	0.706	0.013*	0.041*	0.940	0.065	0.216
<i>Janthinobacterium_571526</i> sp.	-2.857	1.179	0.315	-0.073	-0.149	-0.155	0.048	-0.145	-0.207	0.149	0.849	0.957	0.940	0.890	0.870	0.920	0.896	0.877
<i>Cupriavidus</i> sp.	-0.730	-1.332	-0.517	-0.183	0.996	1.198	-0.329	0.640	1.247	0.853	0.910	0.998	0.980	0.916	0.817	0.984	0.952	0.738
<i>Achromobacter</i> sp.	-4.132	-0.312	0.128	0.092	3.865	4.606	-0.047	5.132	5.377	0.050	0.922	0.937	0.873	0.058	0.025*	0.871	0.013*	0.009*
<i>Pigmentiphaga</i> sp.	-2.783	-0.171	0.097	0.920	0.546	3.394	0.056	2.028	4.754	0.135	0.990	0.977	0.790	0.875	0.050	0.943	0.365	0.005*
<i>Methylopumilus_A_558053 planktonicus</i>	0.520	-2.499	-2.376	-2.379	-2.518	-2.640	-2.395	-2.607	-2.700	0.948	0.537	0.618	0.334	0.231	0.161	0.302	0.182	0.160
<i>Thiobacillus thioparus</i>	8.665	-0.227	0.000	0.513	0.113	-1.368	2.372	-1.103	-3.003	<0.001*	1.000	1.000	1.000	1.000	0.510	0.150	0.717	0.039*
<i>Aquirickettsiella isopodorum</i>	-2.960	1.246	0.362	0.026	-0.139	-0.162	0.190	-0.077	-0.285	0.123	0.836	0.973	0.945	0.884	0.885	0.920	0.901	0.825
<i>Pseudomonadaceae</i> sp.	-1.381	-0.344	-0.945	2.141	4.253	5.651	1.654	3.612	5.410	0.542	0.987	0.938	0.254	0.007*	0.001*	0.490	0.018*	0.001*
<i>Pseudomonas_E_647464</i> sp.	-2.575	1.022	0.151	0.077	-0.037	-0.067	-0.012	-0.035	0.989	0.186	0.911	0.993	0.959	0.932	0.916	0.949	0.932	0.748
<i>Cellvibrio gandavensis</i>	-1.289	-0.061	-0.813	-0.990	-1.168	-1.158	-1.119	-1.133	-1.304	0.593	0.995	0.946	0.782	0.648	0.628	0.740	0.638	0.537
<i>Moraxellaceae</i> sp.	0.529	-2.408	-0.843	-1.219	-2.310	-2.363	-2.140	0.220	-2.395	0.940	0.600	0.979	0.768	0.255	0.216	0.373	0.998	0.159

Table S3– Relative abundance of the 79 ASVs found within the microalgal microbiome samples. The first digit of the sample ID stands for the day it was collected (0 = day 0, 4 = day 4, 7 = day 7), the second (and third in some columns) digit(s) refer to the treatment (C = control, T1 = treatment 1, T2 = treatment 2) and the digit after the comma refers to the replicate (1 = first replicate, 2 = second replicate, 3 = third replicate).

ASVs/Taxon	0C,1	0C,2	0C,3	0T1,1	0T1,2	0T1,3	0T2,1	0T2,2	0T2,3	4C,1	4C,2	4C,3	4T1,1	4T1,2	4T1,3	4T2,1	4T2,2	4T2,3	7C,1	7C,2	7C,3	7T1,1	7T1,2	7T1,3	7T2,1	7T2,2	7T2,3
<i>Brevundimonas</i> sp.	0.977	1.401	0.625	3.314	2.06	0.713	0.782	0.608	0.981	0.441	1.716	0.605	0.284	0.276	0.502	0.149	0.296	0.2	0.228	0.275	0.423	0.06	0.066	0.454	0.081	0.106	0.033
<i>Solibacillus</i> sp.	0	0	0	3.606	1.702	1.583	0.755	0.877	1.89	0	0	0	0	0	0	0.006	0.006	0.008	0	0	0	0	0	0	0.003	0	0
<i>Pseudomonadaceae</i> sp.	0	0	0	0	0	0	0	0	0	0	0.001	0	0.015	0.636	0.096	0.38	3.442	1.543	0	0	0	0.007	0.282	0.02	0.338	1.376	1.772
<i>Comamonas_F_589250</i> sp.	0.001	0	0	0	0	0	0.001	0	0.001	0.158	0.028	0.02	1.242	0.715	0.794	0.85	0.623	0.416	0.102	0.005	0.003	1.354	0.811	0.534	0.488	0.191	0.196
SI02C1 sp010672925	0.056	0.173	0.035	0.181	0.093	0.027	0.097	0.138	0.178	0.308	0.132	0.096	0.779	1.007	0.754	0.462	1.336	0.892	0.228	0.19	0.158	0.13	0.127	0.299	0.059	0.049	0.012
<i>Bosea</i> sp.	0.255	0.68	0.212	0.836	0.246	0.168	0.191	0.417	0.415	0.074	0.242	0.127	0.153	0.185	0.148	0.101	0.2	0.105	0.053	0.492	0.342	0.021	0.018	0.056	0.014	0.014	0.007
<i>Runella zeae</i>	0.005	0.01	0.006	0.055	0.039	0.008	0.011	0.007	0.022	0.254	0.427	0.193	0.314	0.293	0.308	0.008	0.008	0.009	0.603	0.288	0.053	0.57	0.438	0.725	0.086	0.011	0.009
<i>Tenuifilaceae</i> sp.	0.079	0.098	0.058	0.366	0.225	0.082	0.102	0.079	0.142	0.172	0.888	0.413	0.024	0.029	0.04	0.008	0.019	0.017	0.127	0.645	0.389	0.003	0.005	0.039	0.118	0.046	0.05
<i>Sphingomonas_L_486704</i> sp.	0.203	0.175	0.081	0.849	0.496	0.21	0.253	0.21	0.332	0.013	0.035	0.035	0.05	0.037	0.061	0.053	0.099	0.034	0.006	0.035	0.019	0.051	0.034	0.094	0.057	0.039	0.011
<i>Ancylobacter_B_502330</i> sp.	0.099	0.253	0.111	0.268	0.113	0.057	0.039	0.028	0.039	0.049	0.149	0.234	0.051	0.033	0.051	0.042	0.065	0.038	0.123	0.187	0.329	0.022	0.016	0.042	0.034	0.013	0.006
<i>Sphingopyxis</i> sp.	0.004	0.002	0	0.014	0.005	0.004	0.003	0.001	0.001	0.01	0.019	0.065	0.155	0.227	0.111	0.17	0.237	0.087	0.017	0.043	0.09	0.224	0.147	0.213	0.178	0.153	0.083
Cyanobacteriales sp.	0.117	0.15	0.044	0.39	0.109	0.033	0.172	0.112	0.235	0.027	0.117	0.059	0.036	0.027	0.022	0.033	0.04	0.015	0.038	0.14	0.12	0.019	0.012	0.006	0.015	0.005	0.002
<i>Dyadobacter_906144</i> sp.	0.153	0.112	0.05	0.398	0.204	0.079	0.11	0.042	0.047	0.061	0.077	0.212	0.014	0.011	0.024	0.008	0.014	0.012	0.021	0.05	0.334	0.007	0.002	0.005	0.009	0.007	0.006
<i>Thiobacillus thioparus</i>	0.013	0.063	0.022	0.068	0.035	0.01	0.015	0.021	0.025	0.183	0.012	0.026	0.032	0.057	0.023	0.008	0.013	0.02	0.74	0.015	0.337	0.011	0.012	0.028	0.006	0.007	0.002
Chitinophagaceae_966727 sp.	0.017	0.029	0.01	0.044	0.034	0.009	0.015	0.008	0.011	0.06	0.045	0.159	0.112	0.079	0.051	0.027	0.038	0.031	0.042	0.035	0.124	0.297	0.147	0.113	0.127	0.046	0.04
<i>Sphingobium xenophagum</i> sp.	0.003	0.007	0.006	0.015	0.007	0.003	0.002	0.003	0.004	1.3	0.023	0.011	0.005	0.003	0.007	0.004	0.006	0.003	0.295	0.017	0.006	0.001	0.001	0.005	0.001	0.001	0
<i>Panacibacter_956521</i> sp.	0.016	0.029	0.006	0.046	0.038	0.011	0.011	0.009	0.006	0.032	0.234	0.199	0.108	0.085	0.025	0.02	0.049	0.042	0.007	0.306	0.169	0.073	0.027	0.023	0.034	0.016	0.006
<i>Bradyrhizobium</i> sp.	0.055	0.087	0.04	0.242	0.095	0.039	0.059	0.048	0.066	0.169	0.058	0.027	0.045	0.038	0.091	0.015	0.029	0.014	0.07	0.029	0.066	0.012	0.007	0.03	0.012	0.008	0.01
Burkholderiaceae_A_59252 2 sp.	0	0.005	0	0	0	0	0	0	0	0.293	0.001	0.001	0.061	0.058	0.045	0.052	0.06	0.028	0.094	0.003	0.006	0.117	0.118	0.023	0.113	0.052	0.022
<i>Novosphingobium nitrogenifigens</i>	0.015	0.022	0.016	0.108	0.036	0.032	0.015	0.023	0.026	0	0.002	0.001	0.051	0.048	0.075	0.065	0.146	0.081	0	0.003	0	0.016	0.009	0.036	0.019	0.019	0.01
<i>Rhizorhabdus_483366</i> sp.	0.018	0.018	0.006	0.063	0.027	0.014	0.013	0.011	0.013	0.025	0.106	0.042	0.022	0.014	0.025	0.006	0.012	0.006	0.015	0.076	0.049	0.009	0.007	0.013	0.014	0.009	0.005
<i>Flaviumibacter</i> sp.	0.002	0.001	0.002	0.012	0.004	0.002	0.001	0.002	0.002	0.004	0.047	0.018	0.008	0.014	0.004	0.004	0.02	0.015	0.007	0.079	0.151	0.03	0.038	0.027	0.042	0.029	0.035
<i>Devosia_A_502124</i> sp.	0.05	0.067	0.025	0.171	0.058	0.036	0.039	0.019	0.026	0.002	0.004	0.004	0.007	0.004	0.008	0.011	0.012	0.011	0.001	0.002	0.005	0.001	0	0.003	0.013	0.005	0.004
PHOS-HE28 sp902825795	0	0	0	0	0	0	0	0	0	0.001	0.001	0.004	0.006	0.006	0.002	0.001	0.001	0.001	0.045	0.006	0.213	0.081	0.08	0.055	0.011	0.02	0.016
Alphaproteobacteria sp.	0.026	0.035	0.016	0.098	0.032	0.018	0.013	0.017	0.022	0.01	0.011	0.005	0.015	0.011	0.014	0.01	0.019	0.009	0.018	0.018	0.034	0.008	0.01	0.013	0.023	0.009	0.003
<i>Phenylobacterium kunshanense</i>	0.003	0.01	0	0.007	0.003	0.004	0.001	0.002	0.001	0.038	0.006	0.041	0.005	0.006	0.004	0.007	0.011	0.009	0.031	0.022	0.118	0.008	0.009	0.027	0.089	0.029	0.016
Microbacteriaceae sp.	0.003	0.004	0	0.007	0.003	0.001	0.001	0.001	0.001	0.006	0.081	0.019	0.042	0.021	0.018	0.003	0.006	0.006	0.009	0.04	0.07	0.032	0.018	0.015	0.05	0.025	0.009
<i>Reyranella aquatilis</i>	0	0	0	0	0.003	0	0	0	0	0	0	0	0	0	0	0	0	0	0.015	0	0	0.072	0.048	0.1	0.142	0.042	0.013
<i>Novosphingobium capsulatum</i>	0.018	0.048	0.023	0.12	0.063	0.034	0.022	0.042	0.042	0.001	0.004	0.002	0.002	0.002	0	0	0	0	0.001	0.002	0.002	0	0	0	0	0	0
<i>Polymorphobacter multimanifer</i>	0	0.007	0.001	0.006	0.005	0	0	0.002	0.003	0.012	0.025	0.019	0.016	0.014	0.018	0.016	0.027	0.018	0.006	0.11	0.038	0.011	0.007	0.018	0.02	0.01	0.005
<i>Pigmentiphaga</i> sp.	0	0	0	0	0	0	0	0	0	0.001	0	0	0	0	0	0.004	0.007	0.005	0	0	0	0.001	0	0.01	0.269	0.079	0.023
<i>Rhizobium_A_501058</i> sp.	0.024	0.033	0.024	0.065	0.018	0.013	0.016	0.006	0.009	0.002	0.004	0.007	0.012	0.009	0.004	0.005	0.009	0.009	0.002	0.004	0.019	0.002	0.002	0.001	0.002	0.001	0.001
<i>Sphingopyxis terrae</i>	0	0	0	0	0	0	0	0	0	0.002	0.002	0.01	0.014	0.017	0.01	0.012	0.021	0.008	0.004	0.086	0.034	0.02	0.018	0.017	0.005	0.008	0.006
<i>Sphingobium_A_485959</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0.006	0.004	0.009	0.045	0.086	0.069	0	0	0	0.002	0.001	0.007	0.013	0.017	0.008
<i>Dyadobacter jiangsuensis</i>	0.012	0.028	0.009	0.062	0.041	0.013	0.02	0.016	0.03	0.002	0.003	0.002	0.003	0.003	0.002	0.001	0.001	0.003	0	0.002	0.001	0	0	0	0.001	0.001	0.002
<i>Pedobacter_887417</i> sp.	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.001	0	0	0	0	0	0	0.013	0.12	0.023	0.008
<i>Roseomonas_A_507058</i> sp.	0.002	0.023	0.009	0.019	0.016	0.004	0.006	0.006	0.007	0	0.007	0.002	0.001	0	0	0.001	0.001	0	0	0.021	0.003	0.001	0.001	0.001	0.007	0.007	0.003
<i>Variovorax</i> sp.	0	0	0	0	0	0	0	0	0	0.003	0	0	0.022	0.015	0.033	0.008	0.014	0.001	0	0	0	0.004	0.002	0.002	0.012	0.004	0
<i>Mucilaginibacter limnophilus</i>	0.003	0.01	0.009	0.019	0.009	0.001	0.004	0.004	0.004	0.001	0.002	0.001	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0
<i>Achromobacter</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0.001	0.001	0.001	0.004	0.004	0.002	0	0	0	0.012	0.007	0.002	0.015	0.011	0.004
<i>Sphingobium yanoikuyae</i>	0.001	0	0	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0.001	0	0.003	0	0	0	0	0.001	0	0	0

Cupriavidus sp.	0	0	0	0	0	0	0	0	0.005	0	0	0.003	0.001	0	0.003	0.001	0.001	0.001	0	0	0.006	0.002	0	0.007	0.002	0.001			
Sphingobium algorifonticola	0.001	0.008	0.001	0.006	0.006	0.001	0.001	0.001	0.002	0	0.001	0.001	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0			
Rhodobacteraceae sp.	0	0	0.001	0	0	0	0	0	0	0	0.001	0	0	0	0	0	0	0	0	0.008	0.015	0.001	0.002	0	0	0			
Mesobacillus_A_295502 sp.	0	0	0	0.007	0.005	0.003	0.006	0.003	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Novosphingobium_485351 sp.	0	0.003	0.001	0.01	0.002	0.001	0.002	0.002	0.002	0	0	0	0	0	0	0.001	0	0	0	0	0	0.001	0	0	0.001	0	0		
Caulobacter_487784 sp.	0	0	0	0.004	0.001	0	0	0.001	0.001	0.008	0	0.001	0	0.001	0	0.001	0.002	0.001	0.004	0	0	0	0	0.001	0	0	0		
Rhizobiaceae_A_499470 sp.	0	0.001	0	0.001	0	0	0	0	0	0	0.001	0	0.001	0	0.001	0.001	0.001	0.001	0.001	0.005	0.006	0.001	0.001	0.001	0	0	0		
Pseudochrobactrum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.001	0	0	0	0	0	0	0.001	0.008	0.008	0.004		
Devosia_A_501803 sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.002	0.003	0.002	0.003	0.004	0.002	0.002		
Pedobacter chitinilyticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.011	0.002	0.004		
Moraxellaceae sp.	0.005	0.004	0	0	0	0	0	0	0	0	0	0.002	0	0	0	0	0	0	0	0	0	0	0.002	0.001	0.001	0	0	0	
Rhodococcus_C_375578 sp.	0	0	0	0	0	0	0.001	0	0.001	0	0	0	0	0	0	0.001	0.003	0.003	0	0	0	0	0	0	0.001	0.003	0.002		
Pseudomonas_E_647464 sp.	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.009	0		
Methylobacterium jeotgali	0.001	0	0.001	0.001	0.001	0	0	0	0	0.001	0	0.001	0	0	0.001	0	0	0	0.002	0.002	0.001	0	0	0	0	0	0		
Mycobacterium sp.	0	0.008	0.003	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Janthinobacterium_571526 sp.	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Microcystaceae_A_24131 sp.	0	0	0.005	0.004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Blastomonas sp.	0	0	0	0	0	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Methylopumilus planktonicus	0	0.003	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Kaistia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.002	0.002	0.001	0.001	0.001	0.001
Flavobacterium sp.	0	0.003	0	0.004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Cypionkella sp.	0	0.007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Aquirickettsiella isopodorum	0	0	0	0.007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Nanopelagicus sp.	0	0	0.002	0.004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Ferruginibacter sp.	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
UBA1268 sp.	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Patescibacteria sp.	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Paludibacter sp001873635	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Paludisphaera sp001610895	0	0	0	0.001	0.001	0	0.001	0.001	0.001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Flavobacterium tiangeerense	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
UBA6899 sp.	0	0	0.001	0.003	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
UBA4655 sp.	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Flectobacillus fontis	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Rhodoferax_A_585629 sp.	0	0	0.004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Cellvibrio gandavensis	0	0	0.002	0.003	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
UBA11704 sp.	0	0	0	0.004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
SYFI01 sp009694365	0	0	0.004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Hylemonella_582308 sp.	0	0	0.002	0.002	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

Table S4 – Environmental data collected by Vittorio Lim, Mohamed Emran, Alla Silkina and Eva Sonnenschein of the microalgal microbiome samples. N/A used where data was not collected.

Treatment	Day	Average nitrogen concentration (mg/L)	Average ammonia concentration (mg/L)	Average nitrate concentration (mg/L)	Average phosphate concentration (mg/L)
f/2 medium	0	44	-7.794	35.574	10.871
Diluted digestate	0	42	14.011	101.776	19.519
Fully concentrated digestate	0	47	62.706	24.508	28.152
f/2 medium	4	47	-7.072	60.792	4.135
Diluted digestate	4	92	8.053	157.568	9.642
Fully concentrated digestate	4	57	60.872	18.661	16.916
f/2 medium	7	N/A	-15.350	84.754	-2.316
Diluted digestate	7	N/A	-6.267	106.011	3.083
Fully concentrated digestate	7	N/A	44.275	8.265	11.286

Statement of Expenditure

Student Name: Alexandra Gimblett

Student Number: [REDACTED]

Project Title: Finding new biotechnological solutions - Advancing sustainable algal cultivation with beneficial bacteria.

<u>Category</u>	<u>Item</u>	<u>Description</u>	<u>Cost*</u>
<i>DNA Extraction and 16S rRNA sequencing (testing included)</i>			
Equipment	MicroGEM prepGEM bacteria kit (50 preps)	DNA extraction kit for analysing microbiomes and bacterial samples.	£60
Consumables	1.5ml microcentrifuge tubes (approx. 30)	Tubes for extracting DNA and preparing samples for 16S rRNA sequencing (multiple used throughout methodology).	£2.20
Consumables	2ml microcentrifuge tubes (approx. 4)	Tubes for pooling the PCR samples ready to send off for 16S rRNA sequencing.	£0.40
Consumables	0.2ml PCR tubes (approx. 30)	Tubes used for PCR amplification of DNA samples.	£3
Equipment	Nextera XT Index Kit (28 samples)	Kit used to prepare and process samples for 16S rRNA sequencing.	£112
Materials	Platinum™ II Hot-Start PCR Master Mix (56 samples / 2 reactions)	Polymerase used in the PCR amplifications.	£82
Consumables	0.2ml 8 PCR strip tubes (approx. 10)	Strip tubes used for library preparation for 16S rRNA sequencing.	£10
Materials	Earth Microbiome Primers, 515F 806R	Primers used in the first step / first PCR of library preparation for 16S rRNA sequencing.	£20
Materials	Beckman Coulter™ Agencourt AMPure XP (54 microlitres)	Used for cleaning up steps prior to sending off for 16S rRNA sequencing.	£4.20
Equipment	NEBNext® Library Quant Kit for Illumina® (qPCR quantification) (28 samples)	Used to quantify the concentrations of DNA prior to pooling for 16S rRNA sequencing.	£34.16
Incidental costs	16S rRNA sequencing (28 samples)	Method to analyse the bacterial communities in complex samples.	£200
Materials	Thermo Scientific Chemicals Agar powder (10g)	Agar used to make gels for gel electrophoresis to analyse DNA samples.	£2.86
Materials	TAE Buffer, Tris-Acetate-EDTA, 10X Solution, Electrophoresis, Fisher BioReagents Thermo Scientific™ (100ml)	Used to make gels for gel electrophoresis to analyse DNA samples.	£5.18
Materials	GeneRuler 1 kb Plus DNA Ladder (20 microlitres)	Ladder used in gels for gel electrophoresis used as a reference to estimate the size of DNA molecules.	£11.04
Materials	SYBR™ Safe DNA Gel Stain (40 microlitres)	Highly sensitive stain used for visualization of DNA in agarose gels.	£7.70

Consumables	Nuclease free water (1 box)	Used in several different reactions throughout the DNA extractions and 16S rRNA sequencing process.	£15.68
Consumables	Microspec™ 1 Roll Autoclave Tape (1 roll)	Used to close off gel trays while the gels were setting.	£6.14
Consumables	Blue roll	For any spills or general use.	£2.74
Consumables	Fisherbrand™ SureOne™ Low Retention Non-Filtered Pipette Tips, 10 µL, Extended Length (3 boxes)	Used for preparation for 16S rRNA sequencing as there were a lot of small volumes to place in tubes and extended tips are easier to know if liquid has been taken up into the tip.	£11.46
Co-cultivation (testing included)			
Growth Media	Guillards (F/2) Marine Water Enrichment Solution (30ml)	Media used to test growth of microalgae as well as for the 96 well plate co-cultivation experiment.	£3.90
Growth Media	Luria Broth Base (Miller's LB Broth Base), powder (11.5g)	Powder combined with distilled water for bacterial cultivation and growth. Also combined with agar and distilled water to make LB/10 plates.	£1.20
Growth Media	BG-11 growth media (100ml)	Used for testing of microalgal growth.	£5.50
Consumables	Corning™ Disposable Vacuum Filter/Storage Systems (2 units)	To filter liquid growth media.	£19.90
Materials	Thermo Scientific Chemicals Agar powder (40g)	Agar to combine with growth media and make plates for streaking bacteria or microalgae.	£11.44
Consumables	Sterilin™ Loops (approx. 40)	Sterile loops used to streak plates with bacteria or microalgae.	£4.32
Consumables	Gosselin™ Polystyrene Round Petri Plate (approx. 40)	Sterile plates to pour media into and streak bacteria or microalgae onto.	£9.20
Growth Media	TAP liquid growth media (100ml)	Used for testing microalgal growth.	£5.40
Materials	Axenic culture of <i>T. obliquus</i>	Microalgae used in co-cultivation experiment.	£60
Consumables	Nunc™ MicroWell™ 96-Well Microplates (1 unit)	96-well plates used for co-cultivation experiment.	£2.65
Consumables	Nunc™ 15mL Conical Sterile Polypropylene Centrifuge Tubes (approx. 17)	Sterile tubes used to grow bacteria within LB/10 liquid culture for co-cultivation experiment.	£8.10
Consumables	Thermo Scientific™ Finntip™ Filtered Pipette Tips (4 boxes of varying sizes)	Pipette tips used for testing and preparation of co-cultivation experiment.	£109.40
Total			£831.77

*including VAT and delivery where applicable

I hereby certify that the above information is true and correct to the best of my knowledge.


Supervisors Signature

Students Signature

Statement of Contributors

ECS – Dr Eva C. Sonnenschein,

AHG – Alexandra Holly Gimblett,

VL – Vittorio Lim,

TUW – Dr Tamsyn Uren-Webster,

SN – Shirin Naserikarimvand

Contributor Role	Contributors Involved
Conceptualization	ECS
Data Curation	AHG, VL, SN
Formal Analysis	AHG
Funding	N/A
Investigation	AHG, VL
Methodology	ECS, TUW, AHG
Project Admin	ECS, AHG
Resources	ECS
Software	N/A
Supervision	ECS, TUW
Validation	N/A
Visualisation	AHG
Writing – original draft preparation	AHG
Writing – review and editing	ECS, TUW, AHG

Ethics Approval

Finding new biotechnological solutions - Advancing sustainable algal cultivation with beneficial bacteria.

9088

Project Tree

●

[Finding new biotechnological solutions - Advancing sustainable algal cultivation with beneficial bacteria.](#)

1. Research Ethics Application Form

Action Required on Form	Status	Review Reference	Date Modified
No	Approved	1 2024 9088 7918	30/01/2024 11:42

Risk Assessment for Teaching and Research Activities*

Swansea University; FSE: Biosciences

Name Alexandra Gimblett Signature date 30/01/2024

Supervisor* Eva Sonnenschein Signature date 30/01/2024

Activity title DNA Extraction and PCR analysis of microalgae samples Base location (room no.)

Lab 131a

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOD or their nominee)

University Activity Serial # (enter Employee No. or Student No.)

Start date of activity (cannot predate signature dates) 01/04/2024.

End date of activity (or 'on going') 14/07/2024.

Level of worker MRes

Ethics approval number 1 2024 9088 7918

Approval obtained for Biological Hazards and/or GMO Safety Assessment by SU? N/A

Is your project: (circle the appropriate choice A-D)

- A. Laboratory-based only (i.e. you **never** work in the field)
- B. Field **AND** laboratory-based
- C. Field-only based (i.e. you do not have an allocated laboratory space and **never** work in a laboratory)
- D. Desk based (i.e. no field or laboratory base. i.e. you are only allocated office space [if you are a PhD or research member of staff])

For **category A** complete this Risk Assessment template and associated laboratory protocols, and a Training Record form.

For **category B** complete this Risk Assessment template and associated laboratory protocols, a Training Record form, AND either complete the FSE on-line Field Risk Assessment (**for UG, MSc**) or the relevant University-template form (i.e. Red Form- Off Campus Activities & Risk Assessment Form) (**for MRes, PhD, all staff, visitors**)

For **category C** complete this Risk Assessment template (but not the protocol sheets) and the relevant on-line FSE field risk assessment or University-template forms (see B above for details) and complete a Training Record

For **category D** complete the Training Record template **and** this front page.

***N.B. All staff, visitors and students must have risk assessments for their studies in the University. No work can commence until these have been completed. They must be always available for inspection. Some of these may be paper-based but others can be stored electronically.**

Protocol Risk Assessment Form (Laboratory-only)

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #	Title: DNA Extraction and PCR analysis of microalgae samples.	
Associated Protocols #.....	Location and local rules <i>In addition to Good Laboratory Practice, identify any local rules that apply (specific risks and control measures for work in this environment).</i> Lab 131a	
Description of the protocol: Extracting DNA using the MicroGEM prepGEM bacteria kit, and preparing the samples for sequencing (includes PCRs and gel electrophoresis).		
Additional risks and control measures specific to this protocol: Centrifuge used in the DNA extraction (training provided).		
Who or what may be harmed? ☒ Staff/ PG student carrying out the activity ☐ Contractors ☐ Visitors ☐ Cleaners ☐ Maintenance staff ☐ UG student carrying out activity ☐ Other staff/ students in the vicinity	Vulnerable groups present: ☐ U18/ U16 ☐ New or expectant mother ☐ Other: ☐ Environment (via release to air/water/ground, or incorrect disposal)	

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSHH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.) for chemicals to be used and generated	GHS symbols (SH, AT, H, C, Ex, F, O, Env, CG) All that are applicable.	Skin/Eyes Group (SA, SB, SC, SD, SE)	Inhalation Group (A,B,C,D,E)	Quantity	In use dustiness or volatility	Disposal	Primary containment & storage	Other comments: In use factors affecting exposure and special control measures (e.g. <15 mins duration/ frequency/ splash protection only/ hand immersion/ spraying) Safety/ environmental hazards (H2XX/H4XX)
PrepGEM	H, SH	SC	E	1 - Small	Choose an item.	SU chemical disposal. Do not let enter waterways	Tightly closed supplied vessels, store at 4°C, once opened store at -20°C, protect from exposure to light	H334, H317
Green+ Buffer		SA	A	1 - Small	Choose an item.	SU chemical disposal. Do not let enter waterways	Tightly closed supplied vessels, store at 4°C, once opened store at -20°C, protect from exposure to light	H433
Wash+ Buffer	C	SC	C	1 - Small	Choose an item.	SU chemical disposal. Do not let enter waterways	Tightly closed supplied vessels, store at 4°C, once opened store at -20°C, protect from exposure to light	H314
Lysozyme		SA	A	1 - Small	High dustiness	SU chemical disposal. Do not let enter waterways	Tightly closed supplied vessels, store at 4°C, once opened store at -20°C, protect from exposure to light	N/A

Agarose		N/A	N/A	1 - Small	Low dustiness	Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste 2008/98/EC.	Tightly closed. Dry. Storage class (TRGS 510): 11: Combustible Solids	
Tris-acetate-EDTA (TAE) buffer		SC	D	2 - Medium	Choose an item.	Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste 2008/98/EC.	Keep containers tightly closed in a dry, cool and well-ventilated place. Storage class (TRGS 510): 12: Non-flammable liquids in non-flammable packages.	H226, H301, H310, H314, H315, H317, H318, H319, H330, H400, H410, H411
Nucleic acid stain (e.g. SYBR Safe)		N/A	N/A	1 - Small	Choose an item.	SYBR Safe DNA gel stain is not classified as hazardous waste under U.S. Federal regulations (Resource Conservation and Recovery Act (RCRA)). SYBR Safe stain meets the requirements of the U.S. Clean Water Act and the U.S. National Pollutant Discharge Elimination System (NPDES).	Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers. Incompatible with: Strong acids. Strong oxidising agents	
GeneRuler 1 kb Plus DNA Ladder, ready-to-use		N/A	N/A	1 - Small	Choose an item.	The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions	Keep in a dry, cool and well-ventilated place. Keep in properly labelled container. Store at -20 °C.	

						or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.		
Primers (PCR)				1 - Small		Dispose of in accordance with local regulations. Dispose of waste in accordance with environmental legislation. Keep in suitable, closed containers for disposal.	Keep container tightly closed in a dry and well-ventilated place. Store in freezer at -20°C.	
Platinum™ II Hot-Start PCR Master Mix		SC		1 - Small		The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations. Do not empty into drains. Do not dispose of waste into sewer	Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers. Store at -25 °C to -15 °C.	H302, H318, H411
Agencourt® AMPure XP Beads				Small		SU chemical disposal. Do not let enter waterways	Store in cool dry place.	

GHS symbols– SH (serious health hazard), AT (acute toxicity), H (health hazard), C (corrosive), Ex (explosive), F (flammable), O (oxidiser), Env (environment), CG (compressed gas). These should be obtained from chemical SDS documentation. See Appendix (hazard symbols).

Inhalation Group and Skin/Eyes Group- Hazard groups are classified as A/SA (least hazardous) to E/SE (most hazardous). See Appendix for hazard phrases associated with each group. Hazard phrases can be found on chemical SDS documentation.

Dustiness. Low (Pellet- does not break up), Medium (granular or crystalline), High (fine solid or light powder/dust)

Volatility. Low, medium, high, gas. Consider boiling point of liquid and operating temperature.

Disposal e.g. autoclaving of biohazard, SU chemical disposal

Primary containment: e.g. sealed flask, supplied vessel. **Storage:** e.g. secure chemical storage, fridge, freezer, general chemical storage

Note: A **specific DSEAR risk assessment** must be carried out if:

- The work activity involves the use or storage of **flammable, oxidising or corrosive gas cylinders**.
- The work activity is likely to create an **explosive atmosphere** even after the application of controls stated in the chemical risk assessment.
- The work activity involves the **use of explosives**.

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): Fume hood	
Measures taken to eliminate or substitute/reduce: e.g. using less hazardous, less volume of chemicals	
Personal Protective Equipment and all specific control measures: Gloves and lab coat.	
Emergency procedures (include first aid, fire, spillage, communication methods) N.B. full emergency plans for each chemical are detailed in individual Chemical data Sheets If anything is split use blue roll to soak up and ethanol at 70% to sterilize the surface after the liquid has been cleaned.	
Is exposure monitoring required? No	Is health surveillance required? No
Justification and controls for any work outside normal hours (N.B. UG project students cannot work outside normal hours in a laboratory) No working will be conducted outside of normal hours.	
Supervision/training for worker (highlight) N.B. All relevant training forms (e.g. for specific laboratories) should be completed None required Already trained Training required Supervised always	
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.	
<i>Name & signature of worker Alexandra Gimblett</i>	

<i>Name & counter-signature of supervisor....</i>		<i>Date 30/01/24</i>
Date of first reassessment N/A	Frequency of reassessments N/A	

CHEMICAL RISK ASSESSMENT

GB CLP hazard pictograms



Explosive (Symbol: exploding bomb)



Hazardous to the environment (Symbol: environment)



Flammable (Symbol: flame)



Health hazard/Hazardous to the ozone layer (Symbol: exclamation mark)



Oxidising (Symbol: flame over circle)



Serious health hazard (Symbol: health hazard)



Corrosive (Symbol: corrosion)



Gas under pressure (Symbol: gas cylinder)



Acute toxicity (Symbol: skull and crossbones)

Hazard group

A	B	C	D	E
H304, H315, H319, H336 and all H-numbers not otherwise listed	H302, H312, H332, H371	H301, H311, H314, H317, H318, H331, H335, H370, H373	H300, H310, H330, H351, H360, H361, H362, H372	H334, H340, H341, H350
Least Hazardous Substances				More Hazardous Substances
				Special cases
Notes: All phrases/hazard statements now considered a risk on or via the skin.				

Hazard phrases associated with hazard groups A-E (chemicals caused harm by inhalation) and hazard groups SA-SE (chemicals causing harm via skin or eye contact). More information available here:

<https://www.she.stfc.ac.uk/Pages/ChemicalRiskAssessment.pdf>

Risk Assessment for Teaching and Research Activities*

Swansea University; FSE: Biosciences

Name Alexandra Gimblett Signature

date 30/01/2024

Supervisor* Eva Sonnenschein Signature
30/01/2024

date

Activity title Co-Cultivation of bacteria and microalgae Base location (room no.) Lab 120/123

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOD or their nominee)

University Activity Serial # (enter Employee No. or Student No.)

Start date of activity (cannot predate signature dates) 01/04/2024.

End date of activity (or 'on going') 14/07/2024.

Level of worker MRes

Ethics approval number 1 2024 9088 7918

Approval obtained for Biological Hazards and/or GMO Safety Assessment by SU? N/A

Is your project: (circle the appropriate choice A-D)

- E. Laboratory-based only (i.e. you **never** work in the field)
- F. Field **AND** laboratory-based
- G. Field-only based (i.e. you do not have an allocated laboratory space and **never** work in a laboratory)
- H. Desk based (i.e. no field or laboratory base. i.e. you are only allocated office space [if you are a PhD or research member of staff])

For **category A** complete this Risk Assessment template and associated laboratory protocols, and a Training Record form.

For **category B** complete this Risk Assessment template and associated laboratory protocols, a Training Record form, AND either complete the FSE on-line Field Risk Assessment (**for UG, MSc**) or the relevant University-template form (i.e. Red Form- Off Campus Activities & Risk Assessment Form) (**for MRes, PhD, all staff, visitors**)

For **category C** complete this Risk Assessment template (but not the protocol sheets) and the relevant on-line FSE field risk assessment or University-template forms (see B above for details) and complete a Training Record

For **category D** complete the Training Record template **and** this front page.

***N.B. All staff, visitors and students must have risk assessments for their studies in the University. No work can commence until these have been completed. They must be always available for inspection. Some of these may be paper-based but others can be stored electronically.**

Protocol Risk Assessment Form (Laboratory-only)

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #	Title: Co-cultivation of microalgae and bacteria		
Associated Protocols #.....	Location and local rules <i>In addition to Good Laboratory Practice, identify any local rules that apply (specific risks and control measures for work in this environment).</i> Lab 120/123		
Description of the protocol: Plating liquid media, microalgae (<i>Tetrademus obliquus</i>) and bacterial strains (non-hazardous or pathogenic) for co-cultivation analysis. Plating will be conducted under a biosafety cabinet with appropriate PPE. Before and after plating ethanol will be sprayed to clean surfaces and prevent any microbes being left on surfaces. None of the media is considered hazardous or a risk.			
Additional risks and control measures specific to this protocol: Autoclaves used for dissolving and sterilizing media and sterilizing waste for disposal. Autoclave training required (undertaken).			
Who or what may be harmed? ☒ Staff/ PG student carrying out the activity ☐ Contractors ☐ Visitors ☐ Cleaners ☐ Maintenance staff ☐ UG student carrying out activity ☐ Other staff/ students in the vicinity		Vulnerable groups present: ☐ U18/ U16 ☐ New or expectant mother ☐ Other: ☐ Environment (via release to air/water/ground, or incorrect disposal)	

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSHH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.) for chemicals to be used and generated	GHS symbols (SH, AT, H, C, Ex, F, O, Env, CG) All that are applicable.	Skin/Eyes Group (SA, SB, SC, SD, SE)	Inhalation Group (A,B,C,D,E)	Quantity	In use dustiness or volatility	Disposal	Primary containment & storage	Other comments: In use factors affecting exposure and special control measures (e.g. <15 mins duration/ frequency/ splash protection only/ hand immersion/ spraying) Safety/ environmental hazards (H2XX/H4XX)
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			

GHS symbols– SH (serious health hazard), AT (acute toxicity), H (health hazard), C (corrosive), Ex (explosive), F (flammable), O (oxidiser), Env (environment), CG (compressed gas). These should be obtained from chemical SDS documentation. See Appendix (hazard symbols).

Inhalation Group and Skin/Eyes Group- Hazard groups are classified as A/SA (least hazardous) to E/SE (most hazardous). See Appendix for hazard phrases associated with each group. Hazard phrases can be found on chemical SDS documentation.

Dustiness. Low (Pellet- does not break up), Medium (granular or crystalline), High (fine solid or light powder/dust)

Volatility. Low, medium, high, gas. Consider boiling point of liquid and operating temperature.

Disposal e.g. autoclaving of biohazard, SU chemical disposal

Primary containment: e.g. sealed flask, supplied vessel. **Storage:** e.g. secure chemical storage, fridge, freezer, general chemical storage

Note: A **specific DSEAR risk assessment** must be carried out if:

- The work activity involves the use or storage of **flammable, oxidising or corrosive gas cylinders**.

- The work activity is likely to create an **explosive atmosphere** even after the application of controls stated in the chemical risk assessment.
- The work activity involves the **use of explosives**.

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): Fume hood	
Measures taken to eliminate or substitute/reduce: e.g. using less hazardous, less volume of chemicals	
Personal Protective Equipment and all specific control measures: Gloves and lab coat.	
Emergency procedures (include first aid, fire, spillage, communication methods) N.B. full emergency plans for each chemical are detailed in individual Chemical data Sheets If anything is split use blue roll to soak up and ethanol at 70% to sterilize the surface after the liquid has been cleaned.	
Is exposure monitoring required? No	Is health surveillance required? No
Justification and controls for any work outside normal hours (N.B. UG project students cannot work outside normal hours in a laboratory) No working will be conducted outside of normal hours.	
Supervision/training for worker (highlight) N.B. All relevant training forms (e.g. for specific laboratories) should be completed None required Already trained Training required Supervised always	
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. <i>Name & signature of worker Alexandra Gimblett</i>	

Name & counter-signature of supervisor.



.....

Date 30/01/24

Date of first reassessment

Frequency of reassessments

N/A

N/A

CHEMICAL RISK ASSESSMENT

GB CLP hazard pictograms



Explosive (Symbol: exploding bomb)



Hazardous to the environment (Symbol: environment)



Flammable (Symbol: flame)



Health hazard/Hazardous to the ozone layer (Symbol: exclamation mark)



Oxidising (Symbol: flame over circle)



Serious health hazard (Symbol: health hazard)



Corrosive (Symbol: corrosion)



Gas under pressure (Symbol: gas cylinder)



Acute toxicity (Symbol: skull and crossbones)

Hazard group

A	B	C	D	E
H304, H315, H319, H336 and all H-numbers not otherwise listed	H302, H312, H332, H371	H301, H311, H314, H317, H318, H331, H335, H370, H373	H300, H310, H330, H351, H360, H361, H362, H372	H334, H340, H341, H350
Least Hazardous Substances				More Hazardous Substances
				Special cases
Notes: All phrases/hazard statements now considered a risk on or via the skin.				

Hazard phrases associated with hazard groups A-E (chemicals caused harm by inhalation) and hazard groups SA-SE (chemicals causing harm via skin or eye contact). More information available here:

<https://www.she.stfc.ac.uk/Pages/ChemicalRiskAssessment.pdf>

Risk Assessment of An Activity Involving Deliberate Work with Pathogenic Microorganisms or Samples with Potential to Harbour Pathogenic Microorganisms

This risk assessment form should be used to assist in the assessment of risks from an activity involving deliberate work with an infectious or harmful biological agent. The aim of the assessment is to identify those at risk from infection or other harm and the measures required to eliminate or control the risks to human health and the environment to an acceptable level.

Section 1: General Information

1.1 Principal Investigator/ Academic Supervisor:

Name	Faculty
Eva Sonnenschein	Science and Engineering
Email	Date
[REDACTED]	13/03/2024

1.2 Premises where this work will be carried out:

Building	Laboratories	Containment level
Wallace	131a, 120, 123	1

Section 2: Project Information

2.1 Person undertaking this risk assessment (if different from above):

Name	Faculty
Alexandra Gimblett	Science and Engineering

2.2 Project title:

Finding new biotechnological solutions - Advancing sustainable algal cultivation with beneficial bacteria.

2.3 Project Reference Number:

Suggested format YYMMDD, PI initials, BIO (number if more than one in a month)

240313ECS_AG

2.3 Is this proposal an extension of a previously approved project?

Previously approved	☐ Yes ☒ No
If yes, please complete the form emphasising the connection between the original project and this application.	
Previous reference number	

2.4 Brief overview of the work (in layman's terms):

Please describe the project, detailing aims and objectives, significance, and outcomes, indicating how the biological agent will help to achieve the objectives of the project. This description should contain enough detail to help a non-specialist to understand the project.

Microalgae biomass has been utilised for many commercial uses such as biofuel and biofertilizer production, and wastewater treatment. The cultivation of microalgae for these uses has been extensively studied in recent years to increase the productivity of these cultivation systems. To fully understand how microalgal communities grow and evolve, the effects of microalgae-associated bacteria need to be determined. Microalgae and planktonic bacteria often form either mutualistic or parasitic/pathogenic symbiotic relationships. It is understood that these bacteria, when the relationship is mutualistic, can promote the growth of microalgae by providing various nutrients, and increase the microalgal biomass resulting from their cultivation. This project aims to characterize microalgae-associated bacteria and select algal growth-promoting strains using high throughput screening. These bacterial strains could then contribute to large-scale algal cultivation leading to an innovative, sustainable biotech solution. Bacterial DNA will be extracted from microalgae samples collected from the greenhouses at Swansea University using prepGEM Bacteria DNA extraction kit, MicroGem, PBA005. Bacteria strains will be characterized using PCR and sequencing of the 16S rRNA gene. The effects of any identified bacterial strains will be tested in high throughput assays using lab automation. Investigations into the diversity of the microalgal microbiomes cultivated on agricultural waste will also be completed using amplicon sequencing and bioinformatics.

2.5 Is this biological agent going to form part of an undergraduate practical class?

☐ Yes ☒ No

2.6 Faculty Contacts:

Health, Safety and Resilience Advisor

Lorraine Wild and Will Finn-Lewis

Biological Safety Officer

Dr Tamsyn Uren Webster

Section 3: Identification of Biological Hazards

3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):

Name of microorganism	<i>Tetrademus obliquus</i>	<i>Tetraselmis suecica</i>	<i>Thalassiosira pseudonana</i>
Identified as human pathogen on ACDP list ¹	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4

If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	No	No	No
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not a human pathogen	Not a human pathogen	Not a human pathogen
Consequence of infection to humans	Not known	Not known	Not known
Is the microorganism a specified animal pathogen (SAPO ²)	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	No known risk	No known risk	No known risk
Consequence of spread in environment	May cause algal blooms and subsequent oxygen	May cause algal blooms and subsequent oxygen	May cause algal blooms and subsequent oxygen

	depletion in standing water bodies when high nutrient loads are available.	depletion in standing water bodies when high nutrient loads are available.	depletion in standing water bodies when high nutrient loads are available.
Route of transmission for environmental pathogens (including animals)	No known risk	No known risk	No known risk
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	N/A	N/A	N/A
Listed on Schedule 5 ³	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
¹ ACDP Approved List of (Human) Pathogens ² SAPO Pathogens ³ Schedule 5 Pathogens on the Anti-terrorism & Security			

Section 4: Experimental Procedures

4.1 Description of experimental procedures:

Co-cultivation of microalgae species and bacteria (separate risk assessment will be provided) to determine how specific bacterial strains impact the growth of microalgae.

4.2 Quantities used and frequency of use:

This information will enable you to determine the likelihood of exposure and therefore the risks from this particular activity. Please indicate maximum culture volumes at any time shown as multiples of flask volumes to give an idea of scale.

Max. volume per culture/ sample	250 ml	Max. volume per experiment	250 ml
Frequency of experiments	weekly		

Section 5: Measures to Prevent or Control Exposure

5.1 Preventing exposure

a) Could a less hazardous substance (or form of the substance) be used instead? If it can, then it should be used, or justification be given here why it is not being used. COSHH requires substitution with less hazardous materials wherever possible, but there may be good reasons for not using them.

N/A

5.2 Controlling Exposure

b) Containment Level - what containment level is required for this work with regard to COSHH/ SAPO?

☒ 1	☐ 2	☐ 3
c) CL3 only – application for derogation from the following controls (list if relevant and justify):		
d) Will the work be segregated from others not involved in the work and if not, how will they be informed of the hazards?		

5.3 Engineering Controls (Containment & Ventilation)

a) Is a microbial safety cabinet (or isolator for in vivo work) required? These must be used for activities generating potentially infectious aerosols or splashes.	☐ Yes ☒ No
If yes, please state which class and describe underneath what processes will use it:	Class: ☐ I ☐ II ☐ III
If required, what processes require its use?	
b) Specify other local ventilation control	

measures considered appropriate (e.g., downdraft table, isolator):	
c) Will centrifugation be used?	☒ Yes ☐ No
If yes, will buckets and rotors be sealed?	☐ Yes ☒ No
If yes, where will buckets or rotors be opened?	Lab bench or biosafety cabinet (if cultures are required to be axenic)
If yes, how will spillages in the centrifuge be dealt with?	Wipe up with disinfectant and blue roll
d) Will incubators be used?	☒ Yes ☐ No
If yes, what type (e.g., shaking)?	Shaking and temperature controlled.
If yes, how will spillages in the incubator be dealt with?	Wipe up with disinfectant and blue roll
e) Will sharps be used:	☒ Yes ☐ No
If yes, list and justify their use:	
f) Will animals be deliberately infected with these biological agents?	☐ Yes ☒ No
g) Do you require a licence/ permit?	☐ Yes ☒ No
https://staff.swansea.ac.uk/media/tgn002-abps-and-import-permits.pdf	
h) If yes, describe the procedure, control measures and whether shedding of infectious agents by animals is expected?	

5.4 Personal Protective Equipment (PPE):			
Lab coat	Gloves	Eye or face (specify)	Other
☒ Yes	☒ Yes ☐ No	☐ Yes ☒ No	☐ Yes ☒ No
Details:	Details:	Details:	Details:

5.5 Transportation	
a) How will viable material be transported within the laboratory?	In covered flasks and beakers with liquid media. On agar plates.
b) How will viable material be transported locally outside the laboratory?	In covered flasks or beakers with liquid media or on agar plates in secondary container
c) Will viable material be shipped anywhere (off campus)?	☐ Yes ☒ No
If yes, what will be shipped?	
If yes, how will this be shipped (e.g., Category A, Category B, Exempt, Non-hazardous)?	

5.6 Waste Disposal Procedures:

(Disinfectants, concentrations, exposure times, autoclaving procedures, incinerator procedures, include any animal related wastes.)

Waste	Decontamination method (include details on efficacy)	Disposal route e.g., drain/ incineration/ landfill
Liquid waste	Autoclaving (121 C for 15 mins) to kill the microalgae.	Drain.
Solid waste	Autoclaving (121 C for 15 mins) to kill the microalgae.	Autoclaved waste. Incineration.
Sharp waste	In yellow bins as non-hazardous waste.	Contractor for University waste disposal.

5.7 Emergency Procedures

Spillages

- If covered by local rules/ standard operating procedure, please attach.
- If not covered by local rules/ standard operating procedure. Remember to take into account route of exposure

a) Inside primary containment (if relevant e.g., MSC, isolator):	In the event of a spillage, put on the required PPE and stop the source of the spill. It may be appropriate to move equipment (such as the incubator shelf or tip boxes) away from the spill to thoroughly clean the area. Cover the spill with paper towel. Spray the spillage area and any contaminated materials liberally with 5% BioCleanse (or equivalent) and leave to disinfect for at least 5 minutes. After 5 minutes, wipe the area with paper towel, and discard the paper towel in an autoclave bag.
b) Outside primary containment but within the laboratory (secondary containment):	In the event of a spillage inside the lab but outside the primary containment, put on the required PPE and stop the source of the spill. Do not stand near the spill for 5 minutes (or up to 30 minutes for large spills) to allow any aerosols to settle. It may be appropriate to move equipment (such as chairs or waste bins) away from the spill to thoroughly clean the area. Cover the spill with paper towel. Spray the spillage area and any contaminated materials or equipment liberally with 5% BioCleanse (or equivalent) and leave to disinfect for at least 5 minutes. After 5 minutes, wipe the area with fresh paper towel, and discard the paper towel and any contaminated consumables in an autoclave bag.

c) Outside secondary containment (if relevant):	Only small quantities of algal cultures (max 250 mL) are expected to be outside the laboratory, primarily for use on microscopes or moving to incubator. Algae should always be transported within a leak-proof receptacle to contain any spillages during transport. Leaks within the receptacle may be cleaned with % BioCleanse (or equivalent) upon return to the laboratory. Immediately alert nearby personnel and evacuate the area if necessary. Put on appropriate PPE, such as gloves and goggles. Contain the spill using absorbent materials soaked in a suitable disinfectant, ensuring to clean from the edges toward the center to prevent spreading. Dispose of contaminated materials in designated biohazard waste containers and thoroughly disinfect the spill area. Report the incident to supervisory personnel and update safety protocols if needed.
d) Other procedures (e.g., first aid following any accidental exposure, needle stick, etc.):	Notify someone, such as the supervisor, a technician or a First Aid Officer, in the event of an exposure or possible exposure. Affected individuals should always go directly to A&E in the event of an accidental exposure. It may be appropriate to reserve the culture to inform the health care team of the contaminant. The project supervisor or line manager should always be notified. Complete and submit an Adverse Event form to the following link https://staff.swansea.ac.uk/healthsafety/report-it/ Any queries on adverse events, please contact Health and Safety healthandsafety@swansea.ac.uk or sustainability Sustainability@swansea.ac.uk

Section 6: Personnel and Health Issues

6.1 Vaccination

For ACDP 2 or above human pathogens

N/A

Is an effective vaccination available for any of the pathogens associated with this work?	
6.2 Is health surveillance/ health clearance required?	
Staff and postgraduate research students	☐ Yes ☒ No
Taught students (undergraduate and MSc)	☐ Yes ☒ No (Initial health clearance only)
6.3 Identify any groups of workers who may be at increased risk from this material:	
Anyone who might have compromised resistance to disease for any reason should seek advice from the University Occupational Health Service regarding the need for additional precautions. (For example, pregnant workers, young persons under 18, disabled workers, those with pre-existing disease that increases susceptibility).	While these algae are generally considered low-risk, certain groups of workers may still be at increased risk due to specific conditions or sensitivities. These groups include: • Individuals with Allergies or Asthma: Workers with respiratory conditions or allergies may experience irritation or allergic reactions when exposed to algae or its byproducts. • Immunocompromised Individuals: Those with weakened immune systems may be more susceptible to opportunistic infections, even from typically harmless organisms. • Workers with Skin Conditions: Employees with pre-existing skin conditions, such as eczema or dermatitis, might be more prone to irritation or infection upon contact with algae. • Pregnant Workers: Although algae are generally safe, it is prudent to take extra precautions for pregnant workers to avoid any potential, albeit unlikely, complications. • Workers without Proper Training: Individuals who have not been adequately trained in handling biological materials may be at higher risk due to improper handling or lack of awareness of safety protocols. Although algae are generally safe, it is prudent to take extra precautions for pregnant workers to avoid any potential, albeit unlikely, complications.

	Workers without Proper Training:
6.4 Information, Instruction, and Training	
Describe the training that will be given to all those affected (directly or indirectly) by the work activity.	All individuals working in the mentioned labs are given a Lab Induction prior to working on the Laboratory. Relevant COSHH forms and risk assessments should be read and signed before commencing work. The supervisor is responsible for training new researchers and students.

Section 7: Declaration and Approval

7.1 Principal Investigator:

I the undersigned:

- Confirm that all information contained in this assessment is correct and up to date.
- Will ensure that suitable and sufficient instruction, information, and supervision is provided to all individuals working on the activity.
- Will ensure that no work will be carried out until this assessment has been completed and approved, and that all necessary control measures are in place.
- Will ensure that all information contained in this assessment will remain correct and up to date and re-submit for approval if any significant changes occur.
- Work will only be undertaken in appropriate facilities.

Name	Signature	Date
Eva Sonnenschein		21/03/24

7.2 Approval on behalf of the Faculty:

- Approval of Hazard Group 1 only.
- I support the presentation of this proposal to the Biological Hazards and Genetic Modified Organisms Sub-Committee (for ACDP Hazard Group 2-4, SAPO Hazard Group 2-4 and organisms listed on schedule 5).

The person supporting this proposal must not be involved in the project being proposed.

Faculty BSO	Name	Tamsyn Uren Webster
	Signature	
	Date	11/07/2024
University BSO	Name	Andy Lee
	Signature	

	Date	15-07-24
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7.3 Approval on behalf of the University:

- The risk assessment has been reviewed and approved by the Biological Hazards and GMO Sub-Committee.
- Approval of ACDP Hazard Group 2-4, SAPO Hazard Group 2-4 and organisms listed on schedule 5.

Chair of Biological Hazards and GMO Sub-Committee	Name	
	Signature	
	Date	

Section 8: List of Workers Under This Project

List any persons who will be working on this project. They must have access to this risk assessment and other associated risk assessments. Those listed should sign and date to confirm they have read understand the risk assessment.

Full Name	Worker Type					Signature and date
	Staff	PG Research	PG Taught	UG	Other	
Alexandra Gimblett	☐	☒	☐	☐	☐ Details	13/03/24
Eva Sonnenschein	☒	☐	☐	☐	☐ Details	21/03/24 
Anoshanth Jeyasountharan	☐	☒	☐	☐	☐ Details	17/06/24 Anoshanth Jeyasountharan
Ross Williams	☐	☐	☐	☒	☐ Details	09/07/24
	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	

This can also be completed manually and kept as a hard copy in the laboratory – copies must be available for review by Biological Safety Officer/ Biological Safety Advisor.