Effect of iron and magnesium addition on population dynamics and high value product of microalgae grown in anaerobic liquid digestate

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In this study, FeSO4 supplementation ranging from 0 to 4.5 mM, and MgSO4 supplementation ranging from 0 to 5.1 mM were investigated to observe the effect on the population dynamics, biochemical composition and fatty acid content of mixed microalgae grown in Anaerobic Liquid Digestate (ALD). Overall, 3.1 mM FeSO4 addition into ALD increased the total protein content 60% and led to highest biomass (1.56 g L−1) and chlorophyll-a amount (18.7 mg L−1) produced. Meanwhile, 0.4 mM MgSO4 addition increased the total carotenoid amount 2.2 folds and slightly increased the biomass amount. According to the microbial community analysis, *Diphylleia rotans*, *Synechocystis PCC-6803* and *Chlorella sorokiniana* were identified as mostly detected species after confirmation with 4 different markers. The abundance of *Chlorella sorokiniana* and *Synechocystis PCC-6803* increased almost 2 folds both in iron and magnesium addition. On the other hand, the dominancy of *Diphylleia rotans* was not affected by iron addition while drastically decreased (95%) with magnesium addition. This study helps to understand how the dynamics of symbiotic life changes if macro elements are added to the ALD and reveal that microalgae can adapt to adverse environmental conditions by fostering the diversity with a positive effect on high value product.

Microalgae, having no need for arable land, is an attractive source of high value products by their rapid growth. However, the main obstacle to the commercialization of algae-derived products is the high cost of production1. To overcome this high capital investments and operation costs, high-value co-products such as pigments, proteins, lipids, and carbohydrates should be produced to improve the economics of microalgae applications2 along with wastewater treatment, which is a source to obtain nutrients at a low cost. Anaerobic digestion is biodegradation of nutrient rich biomass, which is commonly used for organic matter stabilization and biogas production. Unfortunately, this process leads to produce Anaerobic Liquid Digestate (ALD)3, which is extremely high in ammonia and orthophosphate. Even though direct land application is considered as the most cost-effective solution due to high soil remediation properties in agriculture and reducing the cost of the logistics4, characteristics of the digestion effluent can cause phytotoxic effects in plants and/or contaminate the groundwater5. In this aspect, microalgae can be efficiently grown in liquid digestate and stabilize the effluent without any further treatment.

The effect of macro elements such as nitrogen and phosphorus on microalgae and its biochemical composition has been the focus of research. However, other macro elements such as iron and magnesium play also a critical role in a variety of metabolic pathways important for microalgae. For instance, iron (Fe) is a crucial micronutrient for almost all living organisms because of its role in metabolic processes such as DNA synthesis, respiration, and photosynthesis. It works as a cofactor for enzymes due to its ability to gain and lose electrons6. Magnesium (Mg), on the other hand, occupies a strategic position as the central element of the chlorophyll molecule, and all microalgal species have an absolute need for this element7. Although, the deficiency of iron and magnesium on microalgal growth and photosynthetic efficiency has been investigated, only a few studies focus on the influence

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of iron and magnesium supply on the biochemical composition. They were all in synthetic media, and only for a limited number of species.

Tap water is mostly used for diluting the wastewater, and certain type of bacteria appeared in tap water had been examined for their effects on microalgae growth\(^6\). Richmond and Becker\(^7\) demonstrated that short-term changes in growth conditions can reduce the number of those undesired microorganisms, which is important for non-axenic cultures grown in unsterilized wastewater. Undefined mixed algal culture isolated from nature is a black box that is needed to be enlightened since each undefined culture is unique and specific to its environment. Non-axenic mix microalgae consortia can perform better than unicellular culture with regard to dominancy change due to stress conditions, which prevents the culture loss and lowers the risk of system contamination. However, the mixed consortia should be observed cautiously to assure that microalgae concentration in the consortia remains higher than the bacteria culture to prevent the disappearance of algal cells\(^10\).

Large-scale taxonomic identification has been a challenge for mixed culture composition analysis. Metabarcoding is a novel terminology\(^11\) that has been used for the large-scale taxonomic identification of complex environmental samples\(^12–14\). DNA metabarcoding has been argued to be the next generation tool for detecting mixed species biodiversity in ecological studies and aquatic ecosystems\(^15,16\). Multi-marker metabarcoding, the use of multiple marker regions, is preferred to characterize mixed cultures that include prokaryotes and eukaryotes\(^17–19\). A wide range of prokaryotic and eukaryotic organisms can be identified via 16S rRNA, 18S rRNA and 23S rRNA barcoding analysis. More specific markers are also available such as tufA region, which was found to be an effective marker for the identification of prokaryotic (the cyanobacteria) and eukaryotic algae\(^20\).

The aim of this study was to elucidate how population dynamics changed, and which species were favored by Fe and Mg supplementation. The effect of these elements on biochemical composition of mixed culture and nutrient removal efficiency was also investigated. This study focused on the first attempts to analyze undefined algal microbiome grown in anaerobic digestate, and it will help to understand how the dynamics of symbiotic life changes if macro elements are added to the ALD. A multi-marker metabarcoding approach was used for the characterization of microorganisms in the mixed cultures in anaerobic liquid digestate by analyzing 16S rRNA, 18S rRNA, 23S chloroplast RNA and tufA marker regions. To the best of the authors knowledge, there is no other study that uses multi-marker metabarcoding approach and reports confirmation of results with 4 markers simultaneously for undefined mixed culture of anaerobic digestion, which is important for revealing the molecular diversity in detail. The results of this study will contribute to the efforts to combine digestate treatment with microalgae cultivation for an effective conversion of high strength dark wastewater into high value byproducts.

**Results and Discussion**

**Effect of iron on cell growth and biochemical composition.** The microalgal growth was monitored by measuring the Chlorophyll-a (Chl-a; mg L\(^{-1}\)) and Suspended Solid (SS) concentrations during the experiment due to the dark color of wastewater\(^1\). Many studies also encountered the same problem where recent studies by Marazzi et al.,\(^18\) and Huy et al.\(^19\) reported that measuring OD values for liquid digestate could be challenging due to their strong color.

Iron is an essential nutrient for the survival of all organisms. It is involved in chlorophyll biosynthesis, and it enhances biomass production. Therefore, iron deficiency invariably leads to a simultaneous loss of chlorophyll and degeneration of chlorophyll structure. In order to protect the light-harvesting pigment content, including carotenoid, chlorophyll levels keep decreasing, where iron limited microalgae was reported to have lower pigment concentrations\(^20\). In this study, the increase of iron concentration increased both chl-a and car amount until 3.1 mM FeSO\(_4\), which supported the argument mentioned above. As depicted in Fig. 1, for all iron concentrations, there were 7 days of slower growth for the mixed culture to adapt to the iron supplementation. On the other hand, the control batch had rapid growth since the mixed culture was adapted to ALD for almost 3 years.

After 7 days, rapid increase of chl-a was observed for all batches, and the batch with 3.1 mM FeSO\(_4\) reached maximum chl-a amount (18.7 mg L\(^{-1}\)) after 16 days. For the carotenoid amount, also maximum amount was observed (5.8 mg L\(^{-1}\)) at 3.1 mM FeSO\(_4\) concentration. These parameters indicated that the efficiency of photosynthesis was related to iron nutrition as Rueter et al.\(^21\) mentioned in their study. They explained that iron was also essential for cytochromes, ferredoxin and iron sulfur proteins and absence of it not only reduced the amount of these essential components of photosynthesis but also disrupted their optimal ultrastructural arrangement, which interfered with the function of the photosynthetic apparatus. However, 4.5 mM FeSO\(_4\) concentration did not lead to any further increase of neither chl-a nor car concentrations (Fig. 1). This can be due to the inhibition by iron-replete where excessive levels of essential metals, like iron, can be detrimental to the organisms\(^22\).

Compared with iron limitation studies, only a few experiments have been done under the iron replete conditions, and they were only with pure marine cultures grown in synthetic media. Sasireka and Muthuvelayudham\(^23\) applied various concentrations of FeSO\(_4\), ranging from 10 µM to 50 µM on *Skeletomena costatum* and concluded that 30µM FeSO\(_4\), 7H\(_2\)O resulted in the highest growth rate, which was a very low concentration of ferrous source compared to our study. Moreover, Huang et al.\(^24\) studied the highest final cell densities of three different algae (*Tetraselmis subcordiformis*, *Nannochloropsis oculata* and *Pavlova viridis*) in different concentrations (0.012, 0.12, 1.2 and 12 mM) of ferric ion, and by increasing the ferric ion concentrations from 1.2 to 12 mM, specific growth rates of three microalgae decreased significantly. In their study, the microalgal cultures treated with 0.12 mM iron showed the highest final cell densities. In our study, increasing ferrous concentration from 0 to 3.1 mM enhanced the growth of mixed culture; however, at 4.5 mM, the algal growth ceased. The algal biomass reached 1.06, 1.24, 1.31 and 1.35 mg L\(^{-1}\) when the ferrous concentrations were 0.6, 1.8, 3.1, and 4.5 mM, respectively (Fig. 2). Moreover, nutrient removal was correlated with the algal biomass productivity, whereas the highest nutrient removal for NH\(_4\)N and PO\(_4\)P was observed at 3.1 mM FeSO\(_4\), with 89% and 76% removal efficiency, respectively.

The growth and biochemical content of microalgae is important as a sustainable biological resource. The synthesis and accumulation of energy storage compounds can be enhanced by using appropriate variations in
cultivation conditions. In this study, protein content increased from 34.1% to 54.2% when concentration of iron was increased at 3.1 mM FeSO₄ (Fig. 2). In this study, along with an increase of protein content, the lipid and carbohydrate contents decreased simultaneously. The reason of concomitant decrease in the levels of both carbohydrates and lipids is that their synthesis pathways are related, where the energy is first stored in the form of carbohydrates and then the excess is converted into lipids²⁵. The synthesis of triacylglyceride (TAG) and carbohydrate tends to compete with each other, and which one among those two types of energy storage is produced appears species specific²⁶. Liu et al.²⁷ reported that *Chlorella vulgaris* supplemented with 10 µM iron exhibited a lipid content up to 56.6% of the biomass by dry weight, which was 3–7 fold higher than lower iron concentrations. Moreover, in Ghafari et al.²⁸’s study, *B. sudeticus*, *C. sorokiniana*, *C. vulgaris*, and *E. oleoabundans* showed 10, 60, 18, and 36% increase in lipid content at 4.32 µM Fe presence, respectively. This was expected as Fe increases overall neutral lipid accumulation due to the down-regulation of iron requiring fatty acid desaturase enzymes. In this study, lipid content decreased since dominancy in the culture changed in favor of a low-lipid-yield microalgae species. Overall, 3.1 mM ferrous addition into ALD increased the total protein content 60% along with highest

**Figure 1.** Change in the concentration of (A) Chl-a, (B) Car as a function of time for different concentrations of iron.

**Figure 2.** Biochemical composition and biomass amount of the mixed culture at different FeSO₄ concentrations.
Table 1. FAME profiles for mixed microalgae under different iron concentrations. *SD: Standard Deviation. Bold font indicates statistically significant difference with respect to 0 mg/L Fe concentration (t-test, P < 0.05).

| FeSO4 Concentration (mg L⁻¹) | Saturated fatty acids (SUFA) | Monounsaturated fatty acids (MUFA) | Polyunsaturated fatty acids (PUFA) |
|-----------------------------|-------------------------------|-----------------------------------|----------------------------------|
| 0⁺                          | 16:0 32.4±2.9                 | 15:1 6.6±0.34                    | 18.2 4.1±0.11                    |
|                             | 18:0 4.7±0.22                 | 18:1 n-9t 7.6±0.44               | 18.2 9c 6.8±0.23                 |
|                             |                               | 18:1 n-9c 10.1±1.5               | 18.3 1.3±0.09                    |
|                             |                               |                                  | 20.1 0.26±0.01                   |

Table 2. Impact of different magnesium concentrations on cell growth, nutrient removal efficiencies, and pigments. *SD: Standard Deviation Bold font indicates statistically significant difference with respect to control MgSO₄ concentration (t-test, P < 0.05).

Effect of magnesium on cell growth and biochemical composition. Since magnesium is the center element of chlorophyll, a higher chlorophyll-a amount was expected with increasing MgSO₄ concentrations; however, there was no significant differences on neither biomass amount nor chlorophyll amount (Table 2). However, the highest carotenoid amount between all batches and concentrations was observed at 0.4 mM MgSO₄ concentration (7.4 mg L⁻¹), where it was 3.3 mg L⁻¹ at control cultivation and 5.8 mg L⁻¹ at 5.1 mM MgSO₄ presence. Carotenoids are synthesized in the chloroplast by the action of a series of nuclear-encoded membrane proteins, and Varela et al. mentioned that some microalgae have the ability to accumulate carotenoids under unfavourable conditions.
The highest nutrient removal for NH₃-N and PO₄-P were observed at 0.4 mM MgSO₄ with 88.3% and 69.2% removal efficiency, respectively. Magnesium is one of the chemicals being studied due to its potential for P and N removal in municipal wastewater treatment. Its reaction mechanism is the same as other chemical precipitation processes. Moreover, Huang et al. also reported that Mg²⁺ dominates algal cell absorption and phosphorus utilization. However, in this study, magnesium addition did not improve neither nitrogen nor phosphorus removal compared to control and iron cultivation batches.

The biochemical components of microalgae can be also influenced by the different concentrations of magnesium in the culture medium. However, in this study there were fluctuations between Mg concentration and biochemical composition. The lipid amount increased by 27% when the magnesium concentration was increased from 0 mM to 3.5 mM; however, it started to decrease at 5.1 mM MgSO₄ presence. Huang et al. also observed that the supplementation of 100 μM Mg²⁺ to the culture medium increased the lipid content of Monoraphidium sp. This result indicated that Mg²⁺ has the potential to stimulate lipid accumulation in microalgae but the threshold of the concentration where it starts to cause inhibition should be determined. Ulloa et al. and Sydney et al. also mentioned that the addition of an appropriate concentration of Mg²⁺ to the culture medium increases the lipid content and lipid productivity of microalgal cells, whereas excess Mg²⁺ decreases the lipid content and lipid productivity (Fig. 3).

The fatty acid compositions mainly consisted of C16:0, C18:0, C18:1, and C18:2; however, Mg²⁺ addition decreased the amount of fatty acids under all concentrations. The highest C16:0 was observed at control with 176.2 ppm, where it was 55.1, 43.2, 42.6 and 27.5 ppm for 0.4, 2, 3.5 and 5.1 mM MgSO₄ concentrations, respectively. The fatty acid compositions (ppm) (Mean ± SD*, n = 3) are presented in Table 3.

### Table 3. Fatty acid compositions under different Mg concentrations. *SD: Standard Deviation Bold font indicates statistically significant difference with respect to control MgSO₄ concentration (t-test, P < 0.05).

| MgSO₄ concentration [mM] | Fatty acid compositions (ppm) |  |
|--------------------------|-------------------------------|--|
|                           | C16:0 | C18:0 | C18:1n9c | C18:2 9c |
| Control                  | 176.2 ± 12.1 | 19.09 ± 2.5 | 37.88 ± 5.99 | 55 ± 6.23 |
| 0.4                      | 55.1 ± 3.5 | 3.8 ± 0.44 | 33 ± 4.35 | 22.5 ± 3.22 |
| 2                        | 43.2 ± 5.8 | 2.36 ± 0.18 | 29.64 ± 4.12 | 17.76 ± 1.98 |
| 3.5                      | 42.6 ± 4.7 | 0.86 ± 0.05 | 20.1 ± 3.11 | 14.8 ± 2.12 |
| 5.1                      | 27.5 ± 3.3 | 0.53 ± 0.04 | 5.6 ± 0.76 | 12.4 ± 1.99 |

**Effect of iron and magnesium on microbial composition.** Algal microbiome of mixed culture was measured for the optimum concentration of iron and magnesium, and compared with the control growth. Multi-marker metabarcoding approach was used for microbial community analysis by analyzing four different markers: 16S rRNA, 23S chloroplast rRNA, 18S rRNA and tufA. Photosynthetic microorganisms like cyanobacteria and microalgae have been considered important in the production of valuable co-products along with biofuels in an economically effective and environmentally sustainable way by improving their high value products. In our mixed algae cultures, Diphylleia rotans, Synechocystis PCC-6803 and Chlorella sorokiniana were found to be mostly abundant species, as confirmed by 18S rRNA, 16S rRNA, 23S chloroplast rRNA and tufA marker analyses. When iron supplementation was applied on the mixed algal culture, the abundances of the same dominant species were diminished or increased. The abundance of Synechocystis PCC-6803 and Chlorella sorokiniana increased approximately 1.4 folds and 2.5 folds, respectively, for both 16S and 23S rRNA, while Diphylleia rotans abundance
did not change noticeably. Most abundant microorganisms detected in the mixed cultures were shown in Table 4, and their phylogenetic trees are given in Fig. S2.

The aim of metabarcoding is not only characterizing the communities and biodiversity with high sensitivity, but also detecting the community dynamics, including the interactions between microalgae and other microorganisms. Some studies indicated that presence of bacteria in mixed algal culture can increase algal production and microalgal-bacterial interactions may lead to increased levels of microalgae species and algal production of valuable compounds. Bacteria relationships on algal growth can be mutualistic or parasitic, and knowledge of these mechanisms can be used in order to enhance the algal biomass and high value products.

The algae-bacteria microbiome can have key roles for modulating microalgal populations by promoting microbial growth. In 16 S rRNA analysis, Synechocystis PCC-6803 was identified in the control sample with 3.5% abundance and the dominancy increased more than twice, up to 8.46% when iron was added; and up to 5.26% when magnesium was added. This increase was also reflected on the protein amount of the mixed culture since the dry weight analysis of C. sorokiniana shows that it has 40% protein, 30–38% carbohydrate and 18–22% lipid content. Moreover, bacterial species belonging to Proteobacteria,

| Marker genes | Genus / species | Control | Iron Batch | Magnesium Batch |
|--------------|-----------------|--------|------------|-----------------|
| **16 S rRNA**| Synechocystis PCC-6803 | 20.14  | 27.51      | 20.02           |
|              | Cyanobium PCC-6307 | 5.75   | 0.07       | 0.07            |
|              | Chlorella sorokiniana | 1.79   | 3.93       | 2.84            |
|              | Deschloromonas fungiphilus | 3.14   | 0.01       | 0.01            |
|              | Burkholderiaceae | 2.55   | 0.84       | 3.86            |
|              | Desulfovibrio oxamicus | 2.00   | 0.01       | 0.03            |
|              | Thauera         | 0.00   | 3.28       | 0.94            |
|              | Azospirillum     | 5.02   | 0.01       | 0.03            |
|              | Burkholderiaceae | 2.55   | 0.84       | 3.86            |
|              | Thermomonas fusca | 0.85   | 0.01       | 3.21            |
|              | Physiophaeaeae SM1A02 | 0.10   | 2.39       | 0.08            |
|              | Planctomicrobium piriforme | 0.54   | 2.28       | 1.91            |
|              | Turneriella     | 0.19   | 0.89       | 4.07            |
|              | Microtrichaceae IMCC26207 | 0.09   | 3.16       | 0.31            |
|              | Sediminibacterium | 0.06   | 0.27       | 2.30            |
|              | Gemmatimonas     | 2.18   | 1.14       | 1.04            |
| **18 S rRNA**| Diphylleia rotans | 45.54  | 42.17      | 2.02            |
|              | Trebouxiophyceae | 10.14  | 19.55      | 12.03           |
|              | Leptophyelae sp. WaAra | 7.60   | 0.05       | 0.04            |
|              | Cercoeeoa sp. 1 YG-2013 | 6.57   | 0.18       | 7.06            |
|              | Fungi           | 4.64   | 0.03       | 0.02            |
|              | Poteriochromonas malhamensis | 3.65   | 0.02       | 0.02            |
|              | Charaeaceae sacculatum | 2.66   | 0.24       | 6.62            |
|              | Poteriochlamydeae | 1.92   | 0.02       | 0.00            |
|              | Spongomonas     | 1.58   | 7.52       | 1.27            |
|              | Nuclearia       | 0.25   | 4.91       | 1.36            |
|              | Amoebeoa sp. Pa18 | 0.40   | 0.32       | 18.17           |
|              | Chlamydomonas noctigama | 0.12   | 0.19       | 18.11           |
|              | Paraphysoderma sedebokerense | 0.84   | 0.49       | 13.22           |
| **23 S rRNA**| Synechocystis PCC-6803 | 59.69  | 81.85      | 80.71           |
|              | Cyanobium PCC-6307 | 28.64  | 0.32       | 0.39            |
|              | Chlorella sorokiniana | 2.18   | 5.95       | 5.93            |
|              | Planctomicrobium piriforme | 0.33   | 5.34       | 4.71            |
|              | tufA            | s. Chlorella sorokiniana | 26.72  | 59.09   | 31.23 |
|              |                | c. Chlorophyceae    | 3.85   | 32.16   | 38.06 |

Table 4. Most abundant microorganisms (>2% abundance) in the cultures based on the taxonomic classification by QHIME 2 analysis.
Actinobacteria abundances, respectively. In addition, one
Turneriella Thermomonas fusca in the magnesium added environment, where Planctomicrobium piriforme under magnesium stress with 16S rRNA analysis, however with 23S rRNA analysis, Proteobacteria has emerged with 3.16% abundance in the iron added environment. The amount of was higher of mixed cultures but also wiped out or emerge microorganisms facultatively. species, noctigama found to be abundant in magnesium stress culture with 4.07% and demonstrated that magnesium had a negative effect on species in both control sample and iron added sample, with abundances of 45.54% and 42.17% respectively. abundant with 2.3%.

In 18S rRNA analysis, heterotrophic flagellate Diphylleia rotans was identified as the most dominant algae species in both control sample and iron added sample, with abundances of 45.54% and 42.17% respectively. However, the dominance of Planctomycetes was diminished in magnesium added culture to an abundance of 2.02%, which clearly demonstrated that magnesium had a negative effect on Diphylleia rotans. On the other hand, Chlamydomonas noctigama emerged with 18.11% abundance. Amoeboid species with Paraphysoderma sedebokerense, emerged with 13.22% abundance in response to magnesium addition. These results concluded that macro elements such as iron and magnesium could not only affect the consortia of mixed cultures but also wiped out or emerge microorganisms facultatively. Poterioochromonas malhamensis, which is known to be a microalgal predator, was detected only in the control culture. Poterioochromonas malhamensis can feed on Chlorella sp., which was consistent with the low amount of Chlorella sp. in the control sample. Therefore, one of the reasons of the increase in the dominance of Chlorella sorokiniana species in different environmental stress conditions might be the disappearance of P. malhamensis species. Characium saccatum is another green algae species, which was detected with 2.66% abundance in the control culture and increased up to 6.62% with magnesium addition. However, the abundance of Characium saccatum decreased to almost zero with iron addition. Different amoeboid organisms were also detected in 18S rRNA analysis such as Leptophyridae sp., WaAra were detected with 7.6% dominance in only control sample, while an unclassified Cercozoo sp. 1 YG-2013 was detected both in the control culture and magnesium added culture with 6.57% and 7.06% abundances, respectively; which implied a negative effect of iron on Cercozoo sp. 1 YG-2013. Flagellated protozoa Spongomonas genus and Nuclearia genus were detected with highest abundance in the iron added culture, whereas it was very low at control and magnesium added samples, which indicated a positive effect of iron on Spongomonas genus and Nuclearia genus.

23S rRNA analysis identified Synechocystis PCC-6803 as the dominant species for all conditions with the same amount of increase when iron and magnesium were added. However, the second dominant species Cyanobium PCC-6307 disappeared when iron and magnesium was added as it was also observed in 16S rRNA results which supported the accuracy of results between different markers.

In tufA analysis, a curated database specialized to detect algal species was used which identified the green algae Chlorella sorokiniana as the most dominant algal species with 26.7, 59, and 31.2% relative abundance for control, iron and magnesium batch, respectively.

In conclusion, the effect of iron and magnesium replete on mixed culture can be explained as the acceleration of the growth of protein-rich species, such as Chlorella sorokiniana, due to the higher need of protein synthesis, leading to improve high protein content of the total algal biomass. Moreover, iron and magnesium addition did not increase neither lipid nor carbohydrate and they were both the highest at control batch (Figs. 2 and 3). According to 23S rRNA results, at control batch Cyanobium PCC-6307 relative abundance was 28.64%, where it was 0.32% and 0.39% when iron and magnesium were added, respectively. This big difference on relative abundance of Cyanobium PCC-6307 in different growth media clarified the reason of higher amount of lipid and carbohydrate observed at control batch compared to other batches.

**Principal component analysis.** Principal component analysis (PCA), which is useful for discerning patterns within the species viability data itself, was applied to observe the relation between the measured parameters and the dominant species in different growth conditions.

The PCA components for each culturing condition were plotted in relation to the biochemical composition, pigment composition and relative abundances of microbial species detected in highest amount via each of 16S, 18S and tufA marker analysis. Mostly abundant cyanobacteria Synechocystis PCC-6803 and Cyanobium PCC-6307, heterotrophic flagellate Diphylleia rotans, green microalgae Chlamydomonas noctigama and Chlorella sorokiniana were included in the PCA biplot (Fig. 4).

Iron effect on the mixed culture was shown to be correlated with the protein content as well as with the abundances of Chlorella sorokiniana and Synechocystis PCC-6803, which are both protein-rich species. Magnesium effect, on the other hand, was shown to be mostly correlated with the pigments as well as with the abundance of Chlamydomonas noctigama. The control batch was correlated with the lipid content as well as with the abundance of Cyanobium PCC-6307. The fatty acid (FA) compositions of the mixed cultures were dependent on the
taxonomic abundances and cultivation conditions. PCA identified the abundance of *Cyanobium PCC-6307* as the major source of variability within the control culture. This variability could be mostly explained by the differences in SFU (C16, C18), MUFA (C18:1), and PUFA (C18:2) contents. C18:3 content was shown to be increased in magnesium batch, which was also observed as positively correlated with the magnesium batch sample in the PCA biplot (Fig. 3). Poerschmann *et al.* also reported that polyunsaturated fatty acids, especially linolenic acid (18:3), was the most abundant in their study with *Chlamydomonas* sp.

**Conclusion**

Successful cultivation of microalgae on wastewater, particularly on digestate, requires close monitoring since each wastewater has its own characteristics. In this study, the microbial community profiles and dynamics were first identified via metabarcoding of 16S rRNA, 18S rRNA, 23S chloroplast rRNA and tufA regions. The community profiles changed drastically due to the macro element addition where the differences in the mixed algal community can be helpful for the adaptation to different environmental and growth conditions. 3.1 mM FeSO₄ and 0.4 mM MgSO₄ addition was found to be the optimum concentration with a positive effect on growth and biochemical composition. Moreover, the dynamics of undefined algal microbiome grown in anaerobic digestate showed significant changes and demonstrated how symbiotic life can be changed if macro elements were added to the ALD. Therefore, this algal microbiome might be a solution for both reducing adverse effect of anaerobic liquid digestate and lowering the cost of microalgae production to help commercialisation of algae-derived products.

**Materials and Methods**

**Wastewater collection and analysis.** The liquid digestate was obtained from a full scale plant decomposing the waste mixture of mechanically/manualy separated organic fraction of municipal solid waste (50%), cattle manure (17%), leaching water from solid waste collection vehicles (8%), expired market wastes (4%) and chicken manure (4%). The characterization of ALD was given in a previous work by Ermis and Altinbas. The PerkinElmer Optima 7000 DV ICP (Inductively Coupled Plasma) optical emission spectrometer was used to analyze the wastewater elements using the standard solutions where calcium (Ca) was 58.1 mg L⁻¹, magnesium (Mg) was 19.1 mg L⁻¹, iron (Fe) was 27.8 mg L⁻¹, manganese (Mn) was 3.9 mg L⁻¹, aluminum (Al) was 9.5 mg L⁻¹, silicon (Si) was 46.7 mg L⁻¹, lead (Pb) was 1.1 mg L⁻¹, boron (B) was 5.8 mg L⁻¹, chromium (Cr) was 5.3 mg L⁻¹, cadmium (Cd) was 0.4 mg L⁻¹, nickel (Ni) was 3.1 mg L⁻¹, silver (Ag) was 4.8 mg L⁻¹, sulfur (S) was 619.7 mg L⁻¹, zinc (Zn) was 0.9 mg L⁻¹, Sr was 1 mg L⁻¹ and sodium (Na) was 871.4 mg L⁻¹.

**Isolation and identification of mixed microalgae.** The isolation of mixed culture of microalgae was performed as described in a previous study. Algal cultures were firstly inoculated in M₅ media containing the following components (per liter): KNO₃ (3000 mg L⁻¹), KH₂PO₄ (740 mg L⁻¹), Na₂HPO₄·2H₂O (260 mg L⁻¹), CaCl₂·2H₂O (13 mg L⁻¹), Fe EDTA (10 mg L⁻¹), FeSO₄·7H₂O (130 mg L⁻¹), MgSO₄·7H₂O (400 mg L⁻¹), and 1 mL Micronutrients consisting of Al₂(SO₄)₃·18H₂O (3.58 g L⁻¹), MnCl₂·4H₂O (12.98 g L⁻¹), CuSO₄·5H₂O (1.83 g L⁻¹), and ZnSO₄·7H₂O (3.2 g L⁻¹). Afterwards, mixed cultures was inoculated in diluted ALD (2%) for acclimation. The culture was inoculated into the same diluted wastewater repeatedly and monitored by microscopic observations frequently. Before the beginning of the each batch cultivation, it was assured that the culture was healthy.

**Figure 4.** PCA biplot for various parameters measured for the mixed culture with different growth media conditions.
Isolated wild-type microalgae culture was firstly checked by light microscopy, and mixed culture was morphologically characterized by using microalgae systematics books. Afterwards, next generation sequencing was performed.

**PCR amplification and sequence analyses of 16 S rRNA, 18 S rRNA, 235 rRNA and tufA.** Molecular confirmation of isolates was performed via next generation sequencing of 16S/18S/23S rRNA and tufA marker regions. Genomic DNA from different mixed microalgae culture samples was isolated and high-throughput sequencing analysis was applied to each sample. Targeted amplicon libraries were constructed with universal V4 region primers [515 f (F), 5'-GTGCGCACGMCCGCGGTA-A-3’ and 806r (R), 5’-GGACTACHVHHHTWTCTAAT-3’] for 16S, TARAeuk454FWD1 (F), 5’-CCAGASCYGCGGTAATTTC-3’ and TARAeukREV3 (R), 5’-ACTTTCGTTCTTGATYRA-3’ primers for 18S rDNA, p23Srv_f1 (F), 5’-GGACAGAAAGACCTATGAA-3’ and p23Srv_r1 (R), 5’-TCAGCGTGT-TACCTCCTAGG-3’ primers for 23S rDNA, (F) 5’-TGAAACAGAAMAWGCTACATT-3’ and (R) 5’-CCTTTCNCGAATMGCRAAW-3’ primers for elongation factor tufA. Purified-amplicon libraries were sequenced using an Illumina MiSeq platform (2×300 paired-end reads).

**Bioinformatic analysis.** Sequence data from marker regions were analysed with Quantitative Insights Into Microbial Ecology 2 program (QIIME2 ver. 2019.445). After demultiplexing raw reads with cutadapt plug-in, denoising and generation of amplicon sequence variants (ASVs) were performed using the Divisive Amplicon Denoising Algorithm (DADA2). Denoising step includes chimera detection and removal, sequence error elimination, singleton exclusion and sequence trimming based on sequence quality graph and expected amplicon size.

The resulting sequences were then classified with the SILVA reference database46,47 (132 release of Dec 13, 2017) and tufA database48. For 16S rDNA analysis, SILVA database trimmed to the V4 region (515F/806R) was used for taxonomic classification. Taxonomic bar plots are given in Fig. S1.

After taxonomic classification, alpha and beta diversity analyses among samples were performed via “qiime diversity core-metrics-phylogenetic” function. To allow for a comparison between the analysis of different samples, we used a user-specified sampling depth per sample per marker analysis. The sample sequences were rarefied (sub-sampled) to 45000, 48000, 50000 and 7000 reads for 16S, 18S, 23S and tufA analyses respectively (Table S1).

Microalgae consortia in this study was identified via amplicon sequencing of small subunit of eukaryotic nuclear ribosomal DNA (18S rDNA), small subunit of prokaryotic ribosomal DNA and eukaryotic chloroplast DNA (16S rDNA), large subunit of eukaryotic chloroplast DNA (23S rDNA), and elongation factor EF-Tu (tufA) gene of prokaryotic (cyanobacteria) and eukaryotic algae.

Alpha diversity of microbes, including phylogenetic diversity was tested to document whether the internal diversity differs among different environmental stress conditions, including iron and magnesium stress. For this purpose, several alpha diversity parameters were tested. Alpha diversity indices (observed ASV richness, Shannon diversity, Faith’s phylogenetic diversity, and Pielou’s evenness) of rarefied samples were calculated in QIIME2 with q2-diversity plug-in. Next, maximum-likelihood phylogenetic trees were constructed with “align-to-tree-mafft-fasttree” function of phylogeny plug-in, which uses FastTree2 Next, maximum-likelihood phylogenetic trees were constructed upon masked MAFFT alignment of representative sequences, and rooted phylogenies were inferred via “fasttree” and “midpoint-root” functions of phylogeny plug-in, which uses FastTree2. Phylogenetic trees, constructed using sequences with most abundant ASVs (minimum total feature frequency of 100), were uploaded to the Interactive Tree of Life (iTOL) tool49 for the illustration of the taxonomic composition of the taxa (Fig. S2).

Beta diversity among the three samples was analyzed as well in order to test statistically whether microbial composition differed among the three conditions. Beta diversity metrics were calculated in QIIME 2 with q2-diversity plug-in and beta function. Principal coordinates analysis (PCoA) was employed based on Bray-curtis distance matrix in order to detect the variation in the microbial communities of samples (Fig. S3).

Principal component analysis (PCA) was also performed in MATLAB R2015a. The PCA plot was constructed in order to demonstrate the differential effects of culturing conditions on the mostly detected microbial species identified in the mixed culture. The percent compositions of lipid, protein, carbohydrate and pigments (carotenoid and chlorophyll) under magnesium and iron added cultures, along with the percent relative abundances of detected microbial species was standardized by z-score normalization prior to PCA analysis.

**Experimental design.** The iron and magnesium source and the starting point concentrations were determined based on the synthetic media study by authors (data not shown). Ferrous sulfate (FeSO₄, Molar Mass: 278 g/mol) starting amount was based on the amount available in M₁ media (130 mg L⁻¹) and gradually increased to 1000 mg L⁻¹ of FeSO₄; and magnesium sulfate (MgSO₄, Molar Mass: 246.5 g/mol) starting amount was based on the amount contained in BG-11 media (130 mg L⁻¹) and gradually increased to 1000 mg L⁻¹ of MgSO₄. The mixed algal culture was operated in batch culture with triplicate using 1000 ml Erlenmeyer flask with 800 ml working volume with different doses of FeSO₄ and MgSO₄ concentrations. The molarity calculations showed that the FeSO₄ concentrations were 0.6, 1.8, 3.1, and 4.5 mM (130, 400, 700, and 1000 mg L⁻¹ FeSO₄); and the MgSO₄ concentrations were 0.4, 2, 3.5 and 5.1 mM (75, 400, 700 and 1000 mg L⁻¹ MgSO₄). The best growth dilution ratio was selected as 10% for mixed culture in a previous study by authors (data not shown) and according to the ICP results, the iron amount was negligible for 10% ALD; hence, 10% ALD was assumed as control batch (0 mM concentration) for both iron and magnesium batch.

All experiments were started with 2.5 mg chl-a L⁻¹ and 0.5 ± 0.1 g L⁻¹ algal biomass and harvested at the end of the stationary phase after 16 days by centrifuging at 5000 rpm for 10 minutes. Cultures were kept in an acclimation cabinet under approximately 150 µmol photon m⁻² s⁻¹ continuous illumination measured with a light meter (Hansatech QRT1 Quantitherm), at 25 °C ± 2 °C during the acclimation period and during the experiments.
where continuous light was provided to increase algal growth. All batches were monitored for more days to observe the death phase to confirm that the stationary phase ended.

**Analytical methods.** Nitrogen was measured as Total Kjeldahl Nitrogen (TKN) and Ammonia (NH₃-N); whereas Phosphorus was measured as Total Phosphorus (TP) and Orthophosphate (PO₄). Suspended Solid, TKN, NH₃-N, TP and PO₄-P values were analyzed as mg L⁻¹ according to Standard Methods⁵⁵.

Total protein content was estimated by the method of Lowry⁶² where samples were pre-boiled for 10 minutes with 2 N NaOH in 1:1 ratio (v/v) as a pretreatment. 1 ml of cooled sample was taken and 700 µl of Lowry solution was added. After vortexing, samples were kept in the dark for 20 minutes at room temperature. Folin solution was prepared 5 minutes before the end of 20 minutes, and 100 µl of folin solution was added to the mixture and vortexed. The samples were left in the dark for at least 30 minutes more and read at spectrometer (750 nm) against the distilled water.

Total carbohydrate was determined by Anthrone reagent method⁶⁶ where 1 ml of the sample was mixed with 2 ml of 75% sulfuric acid and 4 ml of anthrone solution, and incubated for 15 minutes at 100 °C. After samples were cooled down, they were read against distilled water at 630 nm by spectrometer.

Total lipids were calculated by a slightly modified version of Bligh and Dyer’s method⁶⁴. Wet biomass containing 100 ± 5 mg was taken and 1.25 ml of chloroform and 2.5 ml of methanol were added. After 20 minutes of shaking, the samples were vortexed with 1.25 ml of chloroform. The samples were vortexed again by adding 1.25 ml of distilled water. After centrifuging the samples at 3000 rpm for 10 minutes, the lower phase was removed and the samples were evaporated at 70 °C in a vacuum oven. The glass containing lipid was kept 1 hour at 105 °C to reach constant weight. Triplicate samples were analyzed and the average values were taken.

Pigment contents were determined via centrifugation of 2 ml of microalgae cells of each strain at 5000 rpm for 5 min. Pellet was taken and suspended with 2 ml methanol (90%). The mixture was incubated in water bath at 80 °C for 5 min. The steps were continued by centrifugation at 10000 rpm for 5 min. The supernatant was transferred and measured by UV-Vis spectrophotometer at 470 nm, 665 nm and 655 nm against the solvent (methanol) blank.

The concentration of chlorophyll a (chl-a), chlorophyll b and total carotenoids (car) were calculated as explained by Sumanta et al.⁵⁹.

For Fatty Acid Methyl Ester (FAME) analyses, Laurens et al.⁶⁰ and El-Shimi et al.⁶⁷ procedures were followed for acid catalyzed in-situ transesterification with 5–10 mg of microalgal biomass. Methyl tridecanoate (C13:0ME) was prepared as an internal standard and 20 µL C13:0 ME (10 mg/ml), 200 µL chloroform: methanol (2:1, v/v), and 300 µL 0.6 M HCl:methanol (methanolic hydrochloric acid) were added on 5–10 mg dry algal samples and incubated for 1 hour at preheated 85 °C heat block. At the end of the incubation, 1 ml of hexane was added and the upper phase was measured by Gas Chromatography (GC). Shimadzu AOC-20i, GC 2010 model Gas Chromatography with CN100 capillary column (Teknokroma, Barcelona, Spain) with a length of 100 m × 0.25 mm and an internal diameter of 0.2 µm film thickness were used. The carrier gas was helium and the hydrogen flow was 40 ml / min whereas air gas flow was 400 ml/min.

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**References**

1. Borowitzka, M. A. High-value products from microalgae—their development and commercialisation. *Journal of applied phyiology*. 25(3), 743–756 (2002).
2. Chew, K. W. et al. Microalgae biorefinery: high value products perspectives. *Bioresource technology*. 229, 53–62 (2017).
3. Zuliani, L. et al. Microalgae cultivation on anaerobic digestate of municipal wastewater, sewage sludge and agro-waste. *International journal of molecular sciences*. 17(10), 1692 (2016).
4. Rico, C. et al. Anaerobic digestion of the liquid fraction of dairy manure in pilot plant for biogas production: residual methane yield of digestate. *Waste management*. 31(9-10), 2167–2173 (2011).
5. Erims, H. & Altinbas, M. Determination of biokinetic coefficients for nutrient removal from anaerobic liquid digestate by mixed microalgae. *Journal of Applied Phyiology*. 1–9 (2018).
6. Ruit, G. R. & Sahoo, S. Role of iron in plant growth and metabolism. *Reviews in Agricultural Science*. 3,1–24 (2011).
7. Farhat, N. et al. Effects of magnesium deficiency on photosynthesis and carbohydrate partitioning. *Acta physiologiae plantarum*. 38(6), 145 (2016).
8. Lian J., Wijffels R. H., Smith H., Sipkema D. The effect of the algal microbiome on industrial production of microalgae. *Microbiology*. 11(5), 806–818, https://doi.org/10.1111/1365-2954.12996.
9. Richmond A., Becker E. W. Technological aspects of mass cultivation—a general outline. In Richmond A (ed) *CRC handbook of microalgal mass culture*. CRC, Boca Raton, pp 245–264 (1986).
10. Stiles, W. A. et al. Using microalgae in the circular economy to valorise anaerobic digestate: challenges and opportunities. *Bioresource technology*. 267, 732–742 (2018).
11. Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C. & Willerslev, E. Towards next-generation biodiversity assessment using DNA metabarcoding. *Mol Ecol*. 21, 2045–50 (2012).
12. Sauvage, T. et al. A metabarcoding framework for facilitated survey of endolithic phototrophs with tufA. *BMC Ecol*. 16, 8, https://doi.org/10.1186/s12898-016-0068-x (2016).
13. Marcelino, V. & Verbruggen, H. Multi-marker metabarcoding of coral skeletons reveals a rich microbiome and diverse evolutionary origins of endolithic algae. *Sci Rep*. 6, 31508, https://doi.org/10.1038/srep31508 (2016).
14. Smith, K. F., Gurjent S. K., Shauna A. M. & Lesley L. Rhodes (2017) Assessment of the metabarcoding approach for community analysis of benthic–epiphytic dinoflagellates using mock communities, *New Zealand Journal of Marine and Freshwater Research*. 51(4), 555–557, https://doi.org/10.1080/00288330.2017.1298632.
15. Evans, N. T. et al. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Mol. Ecol. Res. 16*, 29–41, https://doi.org/10.1111/1755-0998.12433 (2016).
16. Valentini, A. et al. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol*. 25, 929–942, https://doi.org/10.1111/mee.13428 (2016).
17. Fuhahuru, M. S., Nakai, R., Imura, S. & Naganuma, T. Phylotypic Characterization of Mycobionts and Photobionts of Rock Tripe Lichen in East Antarctica. *Microorganisms*. 7, 203, https://doi.org/10.3390/microorganisms7070203 (2019).
18. Marazzi, F. et al. A novel option for reducing the optical density of liquid digestate to achieve a more productive microalgal culturing. *Algal Research* 24, 19–28 (2017).
19. Huy, M., Kumar, G., Kim, H. W. & Kim, S. H. Phototrophic cultivation of mixed microalgae consortia using various organic waste streams towards remediation and resource recovery. *Bioresource technology* 247, 576–581 (2018).
20. Xing, W., Huang, W. M., Li, D. H. & Liu, Y. D. Effects of iron on growth, pigment content, photosystem II efficiency, and siderophores production of Microcystis aeruginosa and Microcystis wesenbergii. *Current microbiology* 55(2), 94–98 (2007).
21. Rüster, J. L., Hutchinson, D. A., Smith, R. W., Unsworth, N. L. Iron nutrition of Trichodesmium. In Marine pelagic cyanobacteria: *Trichodesmium* and other Diazotrophs. *Springer, Dordrecht*. 289–306 (1992).
22. Shanab, S., Essa, A. & Shalaby, E. Bioremoval capacity of three heavy metals by some microalgae species (Egyptian Isolates). *Plant signaling & behavior*. 7(3), 392–399 (2012).
23. Sassireka, G. & Muthukelayudham, R. Effect of salinity and iron stressed on growth and lipid accumulation in Skeletonema costatum for biodiesel production. *Research Journal of Chemical Sciences*. 5(5), 69–72 (2015).
24. Huang, X., Wei, L., Huang, Z. & Yan, J. Effect of high ferric ion concentrations on total lipids and lipid characteristics of Tetradsissussculiformis, Nannochloris osculata and Pavlova viridis. *Journal of applied physiology* 26(1), 105–114 (2014).
25. Ben-Moussa-Dahmen, L. et al. Salinity stress increases lipid, secondary metabolites and enzyme activity in *Amphora subtropica* and *Dunaliella* sp. for biodiesel production. *Bioresource technology* 218, 816–825 (2016).
26. Rizwan, M., Mujtaba, G. & Lee, K. Effects of iron sources on the growth and carboxylate production of marine microalgae Dunaliellalteriolecta. *Biotechnology and Bioprocess Engineering*. 22(1), 68–75 (2017).
27. Liu, Z. Y., Wang, G. C. & Zhou, B. C. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. *Bioresource technology*. 99(11), 4717–4722 (2008).
28. Ghafari, M., Rashidi, B. & Haznedaroglu, B. Z. Effects of macro and micronutrients on neutral lipid accumulation in oleaginous microalgae. *Biofuels*. 9(2), 147–156 (2018).
29. Pandit, P. R., Fulekar, M. H. & Karuna, M. S. L. Effect of salinity stress on growth, lipid productivity, fatty acid composition, and biodiesel properties in Acutodesmus obliquus and Chlorella vulgaris. *Environmental Science and Pollution Research*. 24(15), 13437–13451 (2017).
30. Varela, J. C., Pereira, H., Vila, M. & León, R. Production of carotenoids by microalgae: achievements and challenges. *Photochemistry research* 125(3), 423–436 (2015).
31. Liang, M. New possibilities of magnesius utilization in wastewater treatment and nutrients recovery (2009).
33. Huang, L. et al. Effects of additional Mg 2+ on the growth, lipid production, and fatty acid composition of Monoraphidium sp. FXY-10 under different culture conditions. *Annals of microbiology* 64(3), 1247–1256 (2014).
34. Ulloa, G. et al. Effect of Mg, Si, and Sr on growth and antioxidant activity of the marine microalga Tetra selmis suecica. *Journal of applied phycology* 24(5), 1229–1236 (2012).
35. Sydney, E. D. et al. Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. *Applied Energy* 88(10), 3291–3294 (2011).
36. Parmar, A., Singh, N. K., Pandey, A., Gnanousouou, E. & Madamwar, D. Cyanobacteria and microalgae: A positive prospect for biofuels. *Bioresource Technology* 102(22), 10163–10172, https://doi.org/10.1016/j.biortech.2011.08.030 (2011).
37. Fuentes, J. I. et al. Impact of Microalgae-Bacteria Interactions on the Production of Algal Biomass and Associated Compounds. *Mar Drugs*. 14(5), 100, https://doi.org/10.3390/md14050100 (2016).
38. Choix, F. J., de-Bashan, L. E. & Bashan, Y. Enhanced accumulation of starch and total carbohydrates in algae-immobilized Chlorella spp. induced by Azospirillum brasilense: II. Heterotrophic conditions. *Enzym. Microb. Technol*. 51, 300–309 (2012).
39. Kazama, E. et al. Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. *Environ. Microbiol*. 14, 1466–1476, https://doi.org/10.1111/j.1462-2920.2012.02733.x (2012).
40. Afi, L. et al. Bacterial degradation of green microalgae: Incubation of Chlorella emersonii and Chlorella vulgaris with Pseudomonas oleovorans and Flavobacteriumaquatile. *Org. Geochem.* 25, 117–130, https://doi.org/10.1016/S0146-6380(96)00113-1 (1996).
41. TouloupaKis, E., Ciuciu, B., Benavides, A. M. S. & Torrillo, G. Effect of high pH on growth of Synecocystis sp. PCC 6803 cultures and their contamination by golden algae (*Poteriochromonas* spp.). *Applied microbiology and biotechnology*. 100(3), 1333–1341 (2016).
42. Lizzul, A., Lekuona-Amundarain, A., Purton, S. & Campos, L. (2018). Characterization of Chlorella sorokiniana, UTEX 1230. *Biologia* 7(2), 25 (2018).
43. Ma, M., Gong, Y. & Hu, Q. Identification and feeding characteristics of the mixotrophic flagellate *Poteriochromonas* malhamensis, a microalgal predator isolated from outdoor massive Chlorella culture. *Algal research* 29, 142–153 (2018).
44. Sauvage, T., Schmidt, W. E., Sudia, S. & Fredericq, S. Data from: A metabarcoding framework for facilitated survey of endolithic phototrophs with tufA. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.6cj8h (2016).
45. Poerschmann, J., Spikerman, E. & Langer, U. Fatty acid patterns in Chlamydomonas sp. as a marker for nutritional regimes and temperature under extremely acidic conditions. *Microbial ecology* 48, 78–89 (2004).
46. Bolyen, E. et al. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science (e27295v1). *PeerJ Preprints* (2018).
47. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Opens external link in new window. *Nucl. Acids Res*. 41(D1): D590–D596 (2013).
48. Tilmaz, P. et al. The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. Opens external link in new window. *Nucl. Acids Res*. 42, D643–D648 (2014).
49. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2–approximately maximum-likelihood trees for large alignments. *PloS one* 5(3), e9490 (2010).
50. Letunic, I. & Bork, P. Interactive tree of life (iTOl) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic acids research* 44(W1), W242–W245 (2016).
51. Cropcheck, C. L. et al. Influence of media composition on the growth rate of Chlorella vulgaris and *Scenedesmus acutus* utilized for CO2 mitigation. *American Society of Agricultural and Biological Engineers*. (2012).
52. Ruel, A. (American Public Health Association). Standard Methods for the Examination of Water and Wastewater, 21st ed. Washington, DC, USA (2005).
53. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*. 193(1), 265–275 (1951).
54. Dubois, M. et al. Colorimetric method for determination of sugars and related substances. *Anal Chem.* 28, 350–6 (1956).
55. Bligh, E. G. & Dyer, W. J. A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology*. 37(9), 911–917 (1959).
56. Sumanta, N., Haque, C. I., Nishika, J., Suprakash, R. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by various extracting solvents. *Research Journal of Chemical Sciences*. IJSSN, 2321, 606X (2014).
57. Laurens, L. M. et al. Strain, biochemistry, and cultivation-dependent measurement variability of algal biomass composition. *Algal Research*. 452, 86–95 (2014).
58. El-Shimi, H. I., Attia, N. K., El-Sheltawy, S. T. & El-Diwani, G. I. Biodiesel production from Spirulina-platensis microalgae by in-situ transesterification process. *Journal of Sustainable Bioenergy Systems*. 3(3), 224 (2014).
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Author contributions
H.E. performed the experiments, analysed the data, and prepared the manuscript. T.C and U.G. analysed the data and prepared the manuscript. M.A. supervised the project, analysed the data and prepared the manuscript.

Competing interests
The authors declare no competing interests.

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