Supplementary Information

Media Recipes

KBr was sourced from Sigma-Aldrich, Australia. KCl, $MgCl_2 \cdot 6H_2O$ and $CoCl_2 \cdot 6H_2O$ were sourced from Chem Supply, Australia. Chemicals for the vitamin solution were sourced from Thermo Fisher Scientific, Australia. All other chemicals were sourced from Univar, Australia.

Nutrient Composition of Media

The concentrations of nutrients in the two wastewater streams, f/2 media and seawater are presented in Table S1. Nutrient concentrations in the wastewater were measured using ICP-MS and FIA. Concentrations of minerals in seawater are typical values reported by Turekian [1]. f/2 Media nutrient content was calculated based on values for seawater and the recipe used.

Table S1 *Nutrient concentrations in media streams*

Nutrient	NH_4-rich Wastewater	NO_3-rich Wastewater	f/2 Media	Seawater
Ammonia	1610	0.2	0.35	0.35
Nitrate	0.01	1180	54.76	0.1
Nitrite	0.04	0.07	0.01	0.01
Phosphate	103	61.4	3.42	0
Arsenic	0.008	0.007	**0.0026**	0.0026
Cobalt	0.005	0.006	0.00309	0.00039
Copper	0.006	0.04	0.0034	0.0009
Iron	1.25	0.44	1.0234	0.0034
Manganese	0.135	0.084	0.0499	0.0004
Molybdenum	0.008	0.02	0.0125	0.01

Methane production from wastewater

NO_3-rich industrial wastewater was fed to a culture of bacteria and methanogens to determine whether additional substrates were present for the production of methane. Methanogens and bacteria were cultured in 20 mL vials with 5 mL of black sea media and fed with 1 mL of wastewater or 25 µL of methanol. Results are shown in Figure S1. Comparison of methane output from cultures fed with wastewater and cultures fed with methanol indicated very minimal methane production from feeding with the NO_3-rich wastewater.

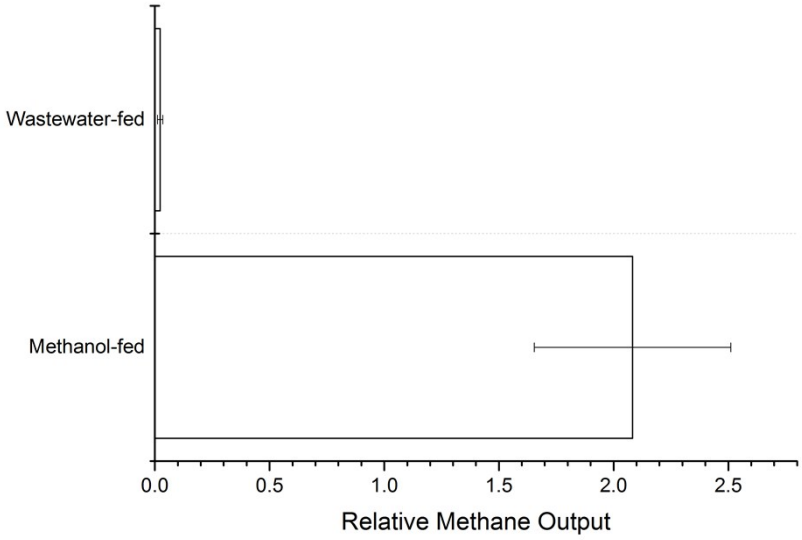

Figure S1 *Methane output from methanogen cultures fed with NO_3-rich wastewater and methanol. There is minimal methane production from methanogen cultures fed with the treated wastewater.*

Cyanobacteria Dry Weight

Dry weights of cyanobacteria cultured in NH4-rich wastewater, NO3-rich waster, f/2 media and seawater were measured after 2 weeks of growth. 10mL samples were filtered through 70 mm glass microfibre filters with pore sizes of 1.0 µm and dried in an oven over 24 hours at 65°C. These dry weights were used to normalise the measured methane output from cyanobacteria grown in each culture medium. The average dry weights for each cyanobacteria culture are shown in Table S2.

Table S2 *Average dry weight of biomass in the cyanobacteria cultures*

Culture	Dry Weight (mg/mL) ± SE
NH_4-rich wastewater	2.007 ± 0.489
NO_3-rich wastewater	2.457 ± 0.418
f/2 media	2.017 ± 0.354
Seawater	2.333 ± 0.463

Light microscopy images of cyanobacteria cultures

After the 2-week cultivation period, cyanobacteria cultured in NH_4-rich wastewater, NO_3-rich wastewater, f/2 media and seawater were observed using light microscopy. A haemocytometer chamber was filled with samples that were diluted by a factor of 4. Samples taken with a 100 µL pipette after mixing the culture by drawing up and expelling liquid with the pipette. Representative images of the cultures are presented in Figure S2. Each image shows the central square of the haemocytometer (1 mm × 1 mm), which had a depth of 0.100 mm, equating to a total volume of 1 µL. Images were taken on a haemocytometer grid with a Leica DM IL

microscope. Similar microscopy observations of cyanobacteria grown in diluted NO_3-rich wastewater were taken using a XX microscope and a representative image is shown in Figure S3.

Microscopy images showed a clear breakdown in the filamentous structure of the cyanobacteria cells in the wastewater cultures. The length of a 22 randomly selected filaments was measured from the images using ImageJ. Filaments had a length ranging from 0.097 mm to 0.329 mm in the f/2 media culture while no filaments longer than 0.007 mm and 0.14 mm could be observed in the NH_4-rich and NO_3-rich wastewaters, respectively. Growth of cyanobacteria in diluted wastewater also produced shorter filaments, with lengths ranging from 0.023 mm to 0.144 mm. Average filament lengths for the cyanobacteria cultures are listed in Table S3.

Table S3 *Average filament length of cyanobacteria cultures*

Culture	**Filament Length (µm) ± SE**
NH_4-rich wastewater	4.5 ± 0.3
NO_3-rich wastewater	54.1 ± 8.0
Diluted NO_3-rich wastewater	73.3 ± 6.2
f/2 media	176.5 ± 15.6
Seawater	152.4 ± 11.8

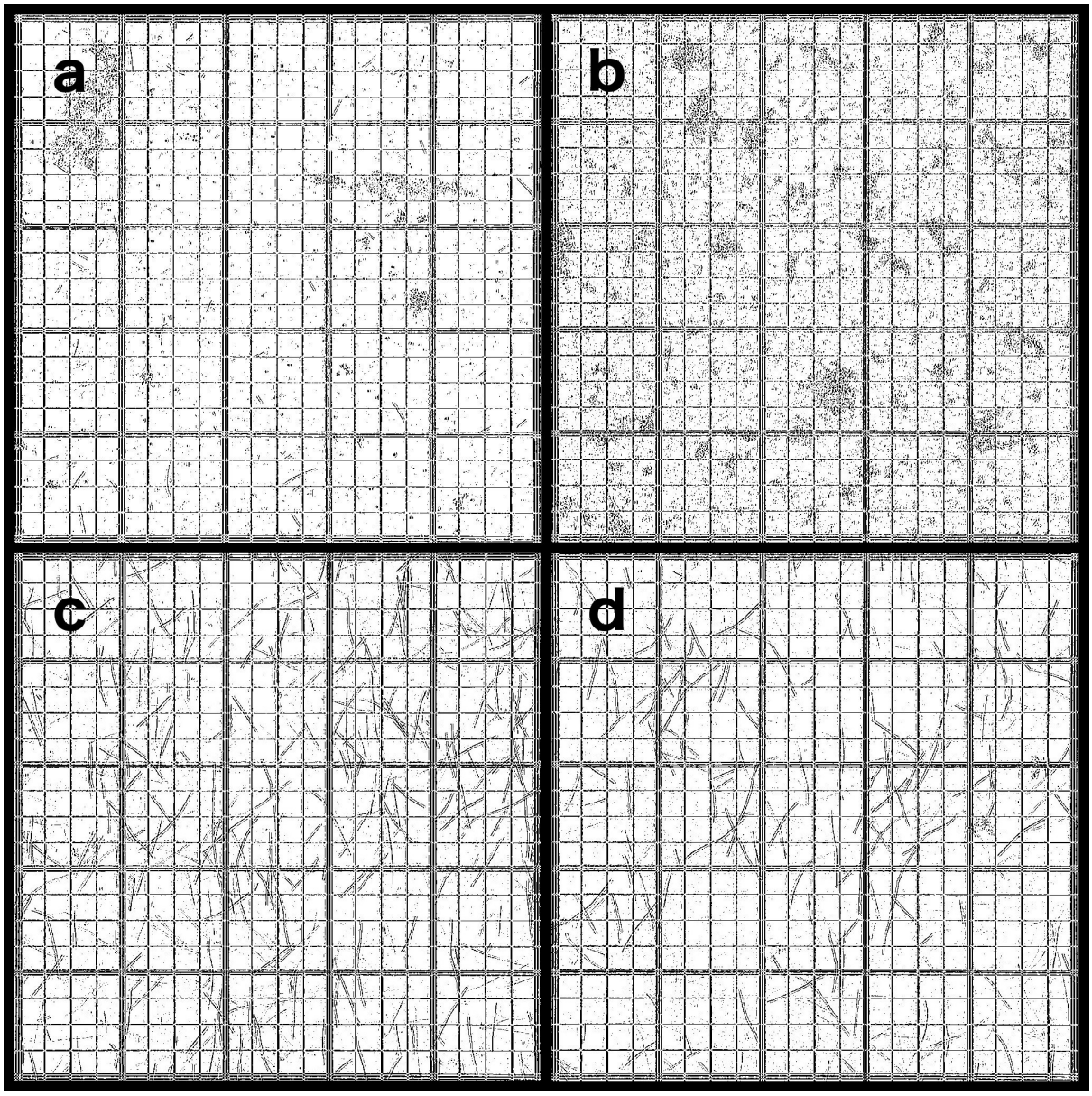

Figure S2 *Images of cyanobacteria cultures after 2 weeks of cultivation. Wastewater cultures showed a visible lack of intact cyanobacteria filaments. The total area of each image is 1 mm^2 and the depth of the cell was 0.1 mm, giving a sample volume of 1 µL in each image. (a: NO_3-rich wastewater; b: NH_4-rich wastewater; c: f/2 media; d: seawater)*

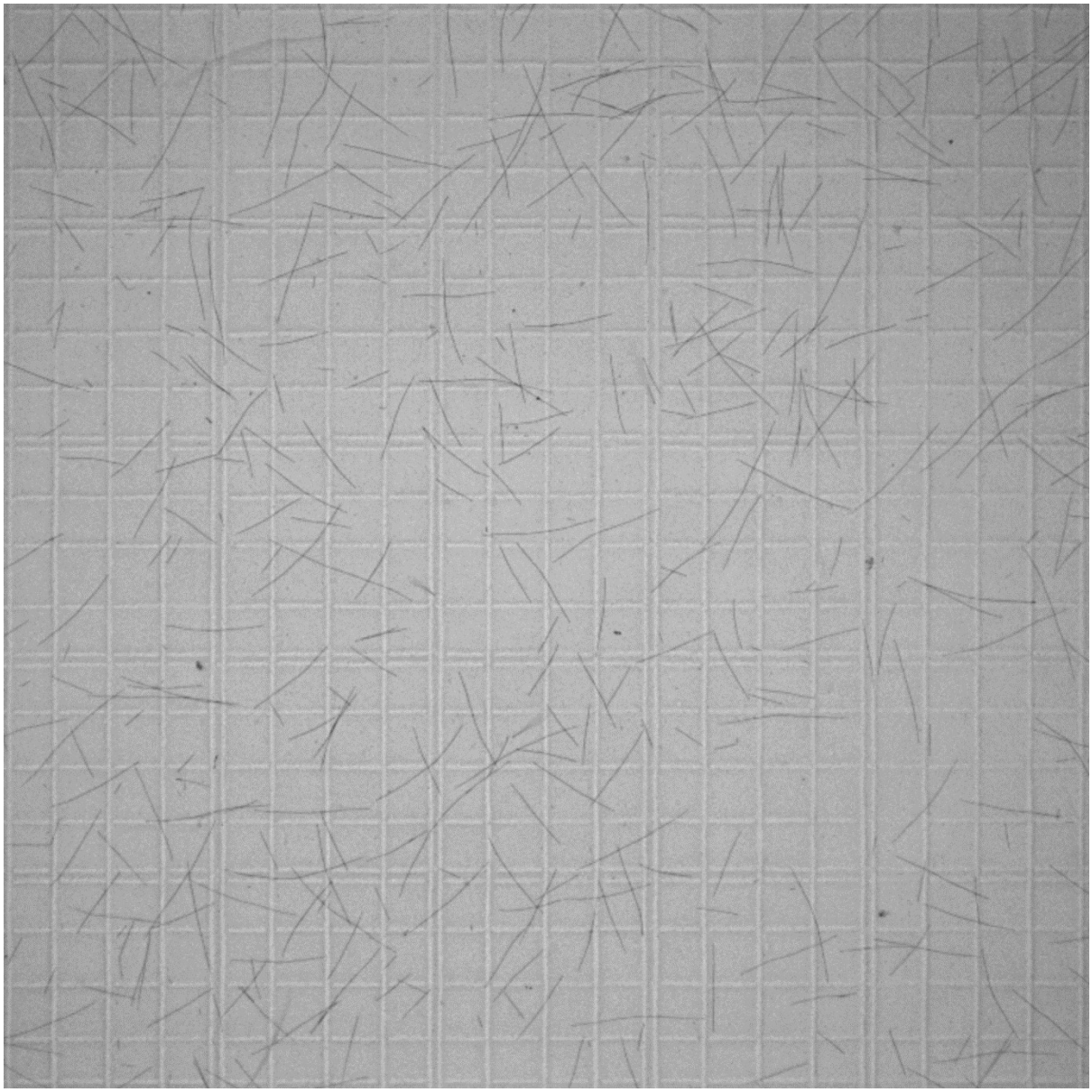

Figure S3 *Image of cyanobacteria after growth in diluted wastewater for 2 weeks. Images showed an abundance of healthy filaments, but lengths were measured to be shorter than for cultures grown in f/2 media. The total area of this image is 1 mm^2 and the depth of the cell was 0.1 mm, giving a sample of 1 µL in the image.*

References

[1] K. K. Turekian, *Oceans*, 2nd ed. Englewood Cliffs, NJ: Prentice-Hall, 1976.