Selection and adaptation of microalgae to growth in 100% unfiltered coal-fired flue gas

Ambreen Aslam, Skye R. Thomas-Hall, Tahira Aziz Mughal, Peer M. Schenk

PII: S0960-8524(17)30240-7
DOI: http://dx.doi.org/10.1016/j.biortech.2017.02.111
Reference: BITE 17683

To appear in: *Bioresource Technology*

Received Date: 9 December 2016
Revised Date: 21 February 2017
Accepted Date: 22 February 2017

Please cite this article as: Aslam, A., Thomas-Hall, S.R., Aziz Mughal, T., Schenk, P.M., Selection and adaptation of microalgae to growth in 100% unfiltered coal-fired flue gas, *Bioresource Technology* (2017), doi: http://dx.doi.org/10.1016/j.biortech.2017.02.111

Selection and adaptation of microalgae to growth in 100% unfiltered coal-fired flue gas

Ambreen Aslam[a,b], Skye R. Thomas-Hall[b], Tahira Aziz Mughal[a] and Peer M. Schenk[b]

[a] Environmental Science Department, Lahore College for Women University, Lahore, Pakistan

[b] Algae Biotechnology Laboratory, School of Agriculture and Food Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia

* Corresponding author email: p.schenk@uq.edu.au, P +61 7 336 58817

Email addresses:

AA: ambreenaslam86@hotmail.com

SRT: s.thomashall@uq.edu.au

TAM: drtahiramughal@gmail.com

PMS: p.schenk@uq.edu.au

Abstract

Microalgae have been considered for biological carbon capture and sequestration to offset carbon emissions from fossil fuel combustion. This study shows that mixed biodiverse microalgal communities can be selected for and adapted to tolerate growth in 100% flue gas from an unfiltered coal-fired power plant that contained 11% CO_2. The high SOx and NOx emissions required slow adaptation of microalgae over many months, with step-wise increases from 10% to 100% flue gas supplementation and phosphate buffering at higher concentrations. After a rapid decline in biodiversity over the first few months, community profiling revealed *Desmodesmus* spp. as the dominant microalgae. To the authors' knowledge this work is the first to demonstrate that up 100% unfiltered flue gas from coal-fired power generation can be used for algae cultivation. Implementation of serial passages over a range of photobioreactors may contribute towards the development of microalgal-mediated carbon capture and sequestration processes.

Keywords

Adaptive evolution; carbon dioxide; carbon sequestration; coal power plant; greenhouse gas emissions; microalgae; microbial community profiling; photobioreactor

1. Introduction

Atmospheric CO_2 levels have now reached the "dangerously high" levels of 400 ppm and future greenhouse gas emissions must be dramatically reduced to mitigate the negative effects of global warming and ocean acidification (Betts *et al.*, 2016). However, the transition to renewable energy sources mostly leads to the generation of electricity, rather than fuels which according to the International Energy Agency directly contributed to 82% of global primary energy supply in 2013 (IEA, 2015). Not considering the costs to the environment and human health, coal-fired power plants are still being commissioned because their cost of electricity generation in relatively low. Efforts are underway to scrub CO_2 from flue gas emitted from pulverized coal power plants using carbon sequestration and storage technology (Supekar *et al*., 2015). However this is estimated to reduce plant thermal efficiency by 11.3–22.9%-points and increase the costs of electricity generation by 63-92% (5.3–7.7 US¢/kWh increase for the levelized cost of electricity (COE) over a 8.4 US¢/kWh COE value for pulverized coal plants without CO_2 capture; Supekar *et al*., 2015).

Carbon dioxide sequestration through biological processes is one of the best known methods for direct conversion of CO_2 into biomass from stationary point source emissions (Stewart & Hessami, 2005). Unicellular microalgae are rapid converters of solar energy to biomass by assimilating atmospheric CO_2 and have therefore been proposed for the treatment of gaseous effluent to reduce CO_2 emissions. One kilogram of dry algal biomass utilizes about 1.83 kg of CO_2 and microalgae reportedly fix waste CO_2 ten times more efficiently than terrestrial plants (Hsueh *et al.,* 2007). Their use as feedstock for bioenergy or their conversion to biochar is well described as a potential method to offset or sequester, respectively, CO_2 emissions. Depending on the strain and its cultivation method, microalgal biodiesel has properties similar to petrochemical diesel, in terms of density, viscosity, flash point, cold flow and heating value (Knothe, 2011; Halim *et al.,* 2012).

Microalgae have a rapid growth potential and many species have oil content in the range of 20–30% dry weight (DW) of biomass (Halim *et al.*, 2012). During the exponential growth phase, they can double their biomass in periods as short as 3.5 h under ideal temperature, light, CO_2 and nutrient availability. This high productivity imparts the potential for high theoretical oil yields in the range of 47,000–308,000 L ha^{-1} $year^{-1}$ (Binaghi *et al*., 2003; Macintyre & Cullen, 2005; Halim *et al.,* 2012). The most significant characteristics of microalgae-based processes is their potential for attaining high solar conversion efficacies by

reducing land and water requirements as compared to plant biomass production (Kadam, 1997). In particular attractive is the fact that algae can grow in almost any type of water (fresh, brackish or seawater) on areas that are not used for agriculture or harbor high biodiversity. Despite their high areal productivity, significant areas of land would still be required to effectively capture CO_2 emissions from power plants. For example, assuming 90% CO_2 uptake efficiency and 20 g DW/m^2 d productivity, 2.73 ha would be required to capture 1 ton CO_2/d, or 224 km^2 (an area of 15 km x 15 km) for a typical 500 MW coal power plant that emits 3 million t CO_2 per year.

Research on CO_2 removal by microalgae covers two fields: (i) the CO_2 capture from flue gases (10–20% CO_2) and (ii) CO_2 uptake capacity of microalgae. In principle, the higher the biomass productivity, the higher the CO_2 uptake. Microalgal culture systems upon feeding with CO_2 lead to mass transfer of CO_2 gas into aqueous media followed by its solubilization in different forms, including CO_2, CO_3^{2-}, HCO_3^- and H_2CO_3 (depending on medium pH) and finally uptake by microalgae during photosynthesis (Van Den Hende *et al.* 2012). Several studies have looked at the potential to cultivate microalgae on actual flue gases or on simulated flue gas mixtures modeled on actual flue stack emissions (McGinn *et al.* 2011). Direct use of industrial flue gas for microalgae cultivation has been demonstrated, in particular when using gas from breweries, cement factories and gas-fired power plants, as these gases are relatively low in SO_x and NO_x (Moreira and Pires, 2016). However, the use of unfiltered flue gas from coal combustion is significantly more challenging, as it can be very high in SO_x and NO_x, microparticles and even heavy metals (e.g. mercury) with high variation between different coal batches. Coal-derived flue gas has been demonstrated to be suitable in principle for microalgae cultivation when diluted with air or added in small quantities (Van Den Hende *et al.,* 2012; Kao *et al.,* 2014; Moheimani 2015). However, flue gas supplementation at higher flow rates rapidly leads to acidification of the growth medium which typically cannot be tolerated by algae. Apart from CO_2, the process variables that influence the success of cultivation are light distribution and saturation, temperature, pH, salinity, nutrient qualitative and quantitative profiles, dissolved oxygen concentration and presence of grazers and growth inhibiting compounds (e.g. heavy metals).

The present study focuses on the mitigation of industrial CO_2 emission from the unfiltered smoke stack of a 4 MW coal-fired power plant through biotransformation into microalgal biomass. Challenges included the presence of small dust particles, and in particular NOx and

SOx that led to a rapid pH decrease when added to the cultivation medium. To overcome this, two mixed microalgal communities had to be slowly adapted over several months and at high flue gas supplementation, a concentration of at least 50 mM phosphate had to be maintained in the medium in the form of phosphate buffer.

2. Material and Methods

2.1 Sampling of environmental microalgal communities

Water samples containing mixed microalgal communities were collected from a University of Queensland (UQ) St Lucia campus fresh water lake (27°30'01.98"S 153°00'58.53"E) at four different points which contained visible algal growth (Freshwater Lake Samples 1-4). In addition, some samples were collected from a storm water creek adjacent to the Sunstate Cement Factory (27°23'.304"S 153°9'.638"E; Stormwater Creek Sample 5), in anticipation that these algae may have preadapted to high carbonate contents. The storm water had been checked with a refractometer for its salinity which shows that it was freshwater. To include brackish and marine algae samples were collected from the tidal Brisbane River (27°29'33.04"S 153°00'07.05"E; Marine Sample 6). Samples were collected in 15 mL non-pyrogenic sterile tubes and examined under a light microscope. Photos of all samples before cultivation are shown in Supplementary Figure 1.

2.2. Cultivation and adaptation of microalgal communities to CO_2 supplementation under laboratory conditions

Mixed cultures from freshwater, stormwater and brackish water were cultivated autotrophically in 250 mL Erlenmeyer flasks with a working volume of 150 mL in Bold Basal Medium (BBM; Nichols, 1973) and f/2 medium (for brackish/marine algae; Guillard and Ryther, 1962) at 25-28°C. As the brackish culture contained some diatoms, 1 mL L^{-1} 0.1 M Na_2SiO_3 was added to f/2 medium supplemented with 100 µM of PO_4^{3-} to bring P contents up to the same level as BBM. The photoperiod was set to 16:8 h light/dark period with LED lights (red, blue and white light) at 110 µmol m^{-2} s^{-1} or with fluorescent lights at 160-170 µmol m^{-2} s^{-1}. Unless otherwise stated, the typical inoculum concentration was 0.1 g DW L^{-1} and cultures were partly shaded to avoid high light stress. Cultures were continuously aerated

with air through bubbling from the bottom of Erlenmeyer flasks with an aeration rate of 11 L min^{-1}. All experiments were carried out in triplicates under batch cultivation.

The optical density (OD) at 440 nm was measured daily to determine growth rates. The pH of the culture was also measured with a pH meter on a daily basis. Nitrate and phosphate levels were also monitored daily until the nutrient levels became undetectable by colorimetric assay (API test kit; Pharmaceuticals) and a UV/VIS spectrophotometer (model U-2800, Hitachi, Tokyo, Japan) at 545 nm and 690 nm, respectively. Microscopic observations were carried out before and after treating with air or various CO_2 concentrations to monitor the difference in populations which was recorded by photography using a Zeiss Axio Fluorescence Microscope.

The six microalgal consortia chosen displayed good growth in the laboratory under conditions of normal aeration and were selected for further experiments involving CO_2 adaptation in fortnightly incremental intervals. Pure CO_2 (provided by a compressed CO_2 cylinder) was used at initially 1%, then 5%, then air for comparison. Culture bubbling was performed by mixing with ambient air at a flow rate 15 L min^{-1} (150 mL CO_2 min^{-1}, then 750 mL CO_2 min^{-1}, respectively). Flowmeters used were 2-20 L min^{-1} (Shun Huan Liu, Lang Yibiao, China) for air and 100-1000 mL min^{-1} (Dwyer Instruments, USA) for CO_2 supplementation.

After cultivation in the presence of air or various CO_2 concentrations, 50 mL samples of algal cells were harvested at 4,700 rpm (4000 x g) by centrifugation for 10 min and pellets were stored at -20ºC for further analyses. This included the sampling of cultures pre-adapted to laboratory-based supplementation with CO_2 for 18S rDNA amplicon sequencing. Following adaptation to 5% CO_2 supplementation, the four samples from the UQ lake were combined to provide a diverse microalgal consortium, now leaving two microalgal communities termed "UQ Lake" and "Storm W.". These pre-adapted cultures provided the starting point for all other subsequent treatments with industrial flue gases under outdoor conditions.

Laboratory experiments with 10% CO_2 supplementation to test the effect of buffering were carried out in larger 500 mL Erlenmeyer flasks with a 250 mL working volume. CO_2 was supplemented by mixing with ambient air at a flow rate of 15 L min^{-1} (1.5 L min^{-1} CO_2 supplementation). In the experiments, combined cultures (UQ Lake + Storm W.) were

cultivated in BBM medium at 28°C with 0.12 g DW L^{-1} initial inoculum with and without the presence of 50 mM phosphate buffer.

2.3. Construction and experimental setup of photobioreactors connected to coal-fired flue gas

The outdoor culture system consisted of twelve 30 L photobioreactors (PBRs) with conical transparent polyethylene (PE) bags (50 cm height and 40 cm diameter) containing 15 L culturing volume and 15 L gas space, each (Figure 1). The bag sleeves were sealed at the bottom in a conical shape where also an inlet for aeration was located in order to prevent cell settling. PBRs were each hung on a steal rod supported by an iron frame. This PBR system contained twelve individual bags, as each of the two microalgal communities had three replicates with and without flue gas supplementation. These were termed F1a, F1b, F1c, and F2a, F2b, F2c (for communities 1 (UQ Lake) and 2 (Storm W.), respectively), with flue gas and A1a, A1b, A1c, and A2a, A2b, A2c, respectively, for air-only control cultures.

Figure 1. Experimental setup of photobioreactors connected to coal-fired flue gas.

Smoke stacks from two 4 MW coal-fired boilers located at Australian Country Choice (ACC) premises in Cannon Hill, Queensland, Australia were used as the source of untreated flue gas. A 1 cm diameter pipe of approx. 15 m length was directly connected to smoke stacks at about 8 m height. An air pump (LP-60, Resun, China) supplied with a foam inlet filter to remove large particles was used to pump flue gas from smoke stack sampling ports. The flue gas was then transferred through pipelines to a gas storage tank (volume: 41.3 L, pressure: 10 kg cm^{-2}) before feeding it to the microalgal PBRs. Each PBR contained an aeration tube, aerators and flow-regulation valves connected by 13 mm PE pipes. This was then fed into a manifold

of 13 mm PE pipe feeder tubes with flow meters (40 L min^{-1} for air and 20 L min^{-1} for flue gas supply), leading to culturing bags. In another air loop, pressurized air only was fed into a manifold of 13 mm PE tubing with a 60 L min^{-1} flow meter. Fast flow was required to achieve even mixing by bubbling for all PBRs. For this reason the 13 mm tubes (one for air and one for air/flue gas mixture) were connected to 4 mm restriction points from which even gas distribution to six PBRs, each, in 4 mm tubes was directed. The flue gas produced from burning of coal was mixed with air using adjustable gas flow meters (60 L min^{-1} for air and 20 L min^{-1} for flue gas supply) to create different v/v mixtures of air-flue gas to the desired concentration. The top of each PBR was tightly sealed except for an outlet port for surplus gas. BBM medium was used for cultivation with municipal water (0.5 ppt salinity). No special requirement of the water was needed but high turbidity was prevented by removing some of the large particulate matter from the flue gas by the foam inlet filter. Sunlight was used as the natural illumination source corresponding to a photosynthetically active radiation (PAR) of 1650.3 µmol photon$\cdot m^{-2}\cdot s^{-1}$ operated on a 12:12 hours light/dark cycle. Experiments were carried out from July-November 2015 with mean temperatures of 21-24^{o}C and solar exposure of 18-21 MJ/m^2. The inlet and outlet loads of flue gas were real-time monitored over several hours by a gas analyzer (AMETEK, Inc., Paoli, PA, USA) to determine the concentrations of CO_2, O_2 and NO.

2.4. Cultivation and adaptation of microalgal communities to industrial flue gas in outdoor photobioreactors

Starter cultures of preadapted microalgal communities (UQ Lake and Storm W.) were grown in 1 L bottles with 150 mL growth medium under continuous fluorescent light for 1 week at pH 7 under laboratory conditions as described above until biomass densities reached 0.15 g DW L^{-1}. These were then transferred to ACC premises and equally distributed into two 30 L PBRs with 15 L growth medium, each, and grown under aeration (10 L min^{-1} per PBR) using 60 L min^{-1} flowmeters (Shun Huan Liu, Lang Yibiao, China). At first, these cultures were treated with air only for about 2 weeks to allow pre-adaptation to outdoor conditions in PBRs and then subsequently mixed and redistributed into two PBRs to commence experiments. However, initial trials with exposure of one of the PBRs for each community to 100% flue gas (containing 11% CO_2) led to a rapid pH change to <2 and death of the cultures. Hence, it was realized that a slow adaptation phase with lower flue gas concentration would be required and the remaining aerated cultures for each community were used for subsequent

experiments. An inoculum density of $OD^{440\ nm}$ around 0.15 was chosen to carry out the PBR-in-series experiments to minimize the toxic effects of flue gas components in sequential reactors, leading to an equal distribution to twelve PBRs, termed F1a, F1b, F1c, and F2a, F2b, F2c (for communities 1 (UQ Lake) and 2 (Storm W.) respectively, with flue gas and A1a, A1b, A1c, and A2a, A2b, A2c, respectively, for air-only control cultures. Flue gas supplementation to PBRs was sequentially increased from 10% (1.1% CO_2), to 30% (3.3% CO_2) over 4 weeks. Unless otherwise stated, when cultures reached $OD^{440\ nm}$ 2.0 they were diluted to $OD^{440\ nm}$ 0.15 which corresponded to 0.1-0.4 g DW L^{-1}, depending on the microalgal populations. Each growth phase was conducted for about 12-16 days before cultures were diluted again. After this adaptation phase to 30% flue gas, 50 mL samples of algal cells were harvested at 4,700 rpm by centrifugation (4000 x g) for 10 min and pellets were stored at -20ºC for DNA extraction followed by 18S rDNA amplicon sequencing.

2.5. Cultivation and adaptation of microalgal communities to 100% industrial flue gas

Flue gas supplementation higher than 30% required buffering and a larger inoculum culture density of 0.3 g L^{-1} to counteract acidification by SOx and NOx in flue gas. Microalgal communities from both sources (UQ Lake and Storm W.) were first combined and then equally distributed into twelve PBRs adjacent to the power plant. Three replicate experiments were carried out for each treatment: with and without the supplementation of high concentration flue gas (air vs flue gas) and with and without the addition of 50 mM phosphate buffer (KH_2PO_4 & Na_2HPO_4) to BBM. Flue gas supplementation to PBRs was then sequentially increased to 50% (5.5% CO_2), and then to 100% (11% CO_2) over a period of 4 weeks. Replicates without buffer included (A1a, A1b, A1c) treated with air and (F1a, F1b, F1c) treated with flue gas. Replicates with buffer included (A2a(P), A1b(P), A1c(P)) treated with air and (F2a(P), F2b(P), F2c(P)) treated with flue gas. Flowmeters used were 2-20 L min^{-1} and 60 L min^{-1} (Shun Huan Liu, Lang Yibiao, China). The initial pH of samples without buffer was around 7 while samples with buffer had a pH around 7.8. All bags were supplied continuously with air-flue gas mixture or air-only at 60 L min^{-1}.

2.6. Sampling protocols

Immediately after inoculation, 3 mL of samples were collected from all bags (flue gas and air) after every experiment for OD, pH, and nutrient (nitrate and phosphate) analyses. Every time after inoculation, sampling was done on the 1st day, 4th day, 8th day, 12th day and 16th day. Some experiments had variations in days as this depended on the period of starvation. In

all experiments, samples were collected until they ran out of nutrients (typically on the 8th day) and then on the 16th day to allow at least 1 week of starvation period for the purpose of lipid analyses. One-liter samples were collected which included 15 mL for DW analysis, 10 mL for biodiversity analysis, 5 mL for lipid (FAME) analysis, 15 mL for protein analysis and remainder for elemental analysis. Samples for lipid analysis were immediately centrifuged (HITACHI, himac CT 6E) at 4,000xg for 10 min and the pellets were preserved at -80^{o}C. Then all samples were freeze-dried and stored at -20^{o}C until being analyzed.

2.7. Cell growth and dry weight analysis

Algal cell growth was estimated by using OD^{440nm} through spectrophotometry. In order to generate a standard curve, each sample was diluted with different dilutions to obtain an absorbance within the range of 0.1-1.0 if the OD^{440nm} was higher than 1.0. The biomass productivity (g DW L^{-1} d^{-1}) was calculated from the variation in the biomass concentrations (g DW L^{-1}) within specific cultivation times (d) by using the following equation (Tang *et al.* 2011):

$$P = (X_1-X_0)/ (t_1-t_o)$$

Where, X_1 and X_0 are the biomass concentration (g DW L^{-1}) in time t_1 and t_0, respectively (Chiu *et.al.* 2008). The biomass concentration (g DW L^{-1}) was used to quantify the cell culture density in each experiment.

The microalgal DW was measured by putting microalgal suspensions in 50 mL pre-weighted nonpyrogenic sterile tubes that were centrifuged at 4,700 rpm for 5 min. After removal of the supernatants tubes with algal pellets were placed in an oven for 48 h at 60^{o}C, and then weighted.

2.8. Fatty Acid Methyl Ester (FAME) analyses

Lyophilized algal biomass (5 mg) was hydrolyzed and methyl-esterified in 300 µL of a 2% (v/v) H_2SO_4 methanol solution for 2 h at 80^{o}C with 50 µg C21:0 (Heneicosanoic acid, SigmaAldrich) as an internal recovery standard. A total of 300 µL of hexane and 300 µL of 0.9 % (w/v) NaCl were added to the mixture. The mixture was vortexed for 20 s and centrifuged at 16,000 x g for 3 min to facilitate phase separation. A total of 150 µL of the hexane layer was injected into an Agilent 6890 gas chromatograph connected to a 5975 MSD mass spectrometer. The running conditions of Agilent's RTL DBWax method (Application

note: 5988-5871EN) was set up as described previously (Lim *et al.* 2012). Fatty acids were identified by comparing retention time with pre-run external standards (37 FAME Mix, Supelco, identified through the NIST library). A linear calibration curve based on the internal standard C22:4(n-6) (adrenic acid) and the internal recovery standard C21:0 were used for fatty acid quantification. Total fatty acid (TFA) content was determined as the sum of all fatty acids. TFA productivity (mg L^{-1} d^{-1}) was calculated as TFA multiplied by the average biomass productivity.

2.9. Isolation of microalgal cultures by plate cultivation and micromanipulation and maintenance

All microalgal communities were regularly examined by microscopy (Zeiss Axioscopic microscope equipped with phase contrast) and recorded by photography. For isolation of pure cultures, either streak plating on petrie dishes containing BBM and agar or micromanipulation was used by isolating single cells under the microscope (Duong *et al.* 2012). Cultures (including polycultures) are maintained by allowing them to grow slowly in jars half filled with air under low light conditions without shaking at 20°C with partial (50%) replacement of the medium at monthly intervals. For polycultures, the culture composition under these conditions may still change but it is anticipated that this process is slow enough so that most strains are still well represented.

2.10. Identification of microalgal strains

After isolation and cultivation of pure cultures, microalgal total DNA was extracted by using an ISOLATE II Plant DNA Kit (Bioline). After extraction, DNA yield and purity were determined spectrophotometrically by a NanoDrop ND-1000 spectrophotometer. Genomic DNA within the 18S rRNA region was amplified by a PCR machine using the following primers: Forward 5′ GCGGTAATTCCAGCTCCAATAGC3′ and Reverse 5′ GACCATACTCCCCCCGGAACC-3′. The ITS1-5.8S-ITS2 region in rDNA was amplified using the primers: ITS forward1: ‘‘ACCTAGAGGAAGGAGAAGTCGTAA’’ and ITS reverse1: ‘‘TTCCTCCGCTTATTGATATGC’’. The PCR product amplified from DNA of each isolate was confirmed as a single band by agarose gel electrophoresis. Sanger DNA sequencing was carried out using the primers above by the Australian Genome Research Facility (AGRF) at The University of Queensland. The sequences were aligned to 18S rDNA sequences obtained from the National Centre of Biotechnology Information Database using BLAST searches. Phylogenetic trees were inferred from the aligned sequence data using the

neighbor joining (maximum likelihood) method (Saitou & Nei, 1987) in Mega5, with the tree being tested by bootstrapping with 1,000 replicates.

2.11. Protein analyses

Protein contents in the algal biomass were measured following the protocol described by López *et al.* (2010) with modifications. A total of 10 mg of crushed, lyophilized algal biomass was suspended in 10 mL lysis buffer (5 mL L^{-1} of Triton X-100, Chem Supply; 0.37 g L^{-1} ethylenediaminetetraacetic acid disodium salt, Chem Supply; 0.03 g L^{-1} phenyl methyl sulfonyl fluoride, Sigma-Aldrich) and incubated for 30 min. Then 100 µL 0.05 g L^{-1} of sodium dodecyl sulfate salt (Sigma-Aldrich) solution was added to 100 µL of the lysis buffer mixture before extracting proteins with a CB-X Protein Assay Kit (G Biosciences) according to the manufacturer's instructions. A microplate photometer (Glomax Multi Detection System, Promega) was used to obtain the absorbance at wavelength 600 nm. Bovine serum albumin (BSA) was used as a standard.

2.12. Biodiversity analysis by 18S rDNA amplicon sequencing

Microalgal communities (UQ-Lake & Storm W.) after initial adaptation to 1% pure CO_2 in the laboratory were compared to the same cultures after adaptation for several weeks under outdoor conditions with supplementation of 10% (1.1% CO_2) of unfiltered flue gas from coal combustion and control samples of the same cultures grown in parallel without any flue gas exposure. This resulted in six cultures that were grown in parallel as triplicates: Lab-UQ Lake and Lab-Storm W., Flue-UQ Lake, Flue-Storm W., and Air-UQ Lake, Air-Storm W., respectively. Each of the replicates (total of 18 samples) was subjected to biodiversity analysis by 18S rDNA amplicon sequencing. All kits and procedures outlined above were used for DNA extraction, quantification and quality control, followed by PCR amplification and purification before Illumina sequencing was performed by AGRF. The PCR products from freshwater lake samples were combined prior to using the same PCR cleanup kit. Only extracted DNA samples with high 260/280 ratios (1.80 to 2.06) were considered for Illumina sequencing. Microalgae were identified up to genus level and wherever possible up to species level using BLAST searches in Genbank and suitable keys (APHA, 1998). Eukaryotic sequences not belonging to microalgae were excluded from the analysis.

2.13. Diversity indices for representative samples

Two diversity indices, Shannon and Simpson, were used for diversity profiling of the phytoplankton (Chaturvedi *et.al.* 1999). The diversity indices were calculated following Shannon and Weaver (1949) and Simpson (1949). Evenness was also calculated following Pielou (1966).

- **Simpson Index (D) -** a measurement that accounts for the richness and the percent of each species from a biodiversity sample within a local aquatic community. The Simpson index is a dominance index because it gives more weight to common or dominant species.

  $$H' = \sum (ni/N)^2$$

  Where, H'= Simpson's index Simpson's index of diversity = 1 – H'
- **Shannon diversity index -** Similar to the Simpson's index, this measurement takes into account species richness and proportion of each species within the local aquatic community. The Shannon index is an information statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled..

  $$H = -\sum(pi)\log_2 pi$$

  Where, H = Shannon diversity index

  pi = ni/N where ni being the number of individual of i^{th} species and

  N= total number of individuals in the sample.
- **Evenness -** Evenness (E) is a measure of how similar the abundance of different species are. When there are similar proportions of all species then evenness is one, but when the abundances are very dissimilar (some rare and some common species) then the value increases. Using the same log base as with H, evenness is defined as:

  $$J = H/\log_2(S)$$

  Where, J = Evenness H = Shannon diversity index S = Species richness

3. Results

Flue gas composition from the 4 MW coal-fired boiler at Australian Country Choice used in the present study included 11.24% CO_2, 8.26% O_2, 388.8 mg/Nm^3 CO, 423.9 mg/Nm^3 NOx (expressed as NO_2), 781.8 mg/Nm^3 SOx (expressed as SO_2, including 2.8 mg/Nm^3 SO_3), and 0.4 mg/Nm^3 particulates.

Exposure of culture medium to 100% flue gas led to a rapid pH drop to 2.3. Therefore, the challenge of this study was not only to identify microalgal strains that are able to rapidly convert dissolved CO_2 into biomass, but to find those that are also highly tolerant to low pH values and continuous exposure to other coal combustion flue gas components, including SO_x and NO_x. Preliminary cultivation experiments in open raceway ponds of several highly productive microalgal strains from the authors' microalgae collection (e.g. *Scenedesmus dimorphus* NT8c, *Chlorella vulgaris*, *Limnothrix planctonica*) showed that supplementation with small amounts of CO_2 (e.g. by using CO_2 to stabilize the pH) led to a near tripling in biomass productivity compared to air-only cultivation, under these carbon-limiting conditions. However, none of these cultures displayed the low pH tolerance that would be required when exposed to flue gas from coal combustion. Therefore, it was hypothesized that such a trait would require careful selective breeding or adaptation over many generations. For this approach mixed biodiverse microalgal communities were chosen to maximize the genetic pool and thus chances to select, identify and adapt strains that are (1) displaying rapid growth and high biomass productivity which is directly related to CO_2 uptake and (2) low pH/high SO_x and NO_x tolerance.

3.1. Initial adaptation of mixed microalgal consortia and diversity analysis

Initially, six mixed microalgal cultures (four from a freshwater lake, one from a cement factory run-off creek, one from tidal brackish water) were pre-adapted to pure CO_2 supply lines under indoor (laboratory-facility) conditions with incremental 1% and 5% and 10% CO_2 concentrations over several weeks. However, the brackish sample was not able to withstand exposure to 5% CO_2 and therefore had to be excluded early from subsequent experiments, while the other freshwater cultures stopped growing when exposed to 10% CO_2. Fourteen distinct pure cultures were isolated from the freshwater samples and identified by morphology and 18S rDNA sequencing (Figure 2). Following BLAST homology searches, cultures displayed the highest matches to *Chlorella* sp. KF144172.1, *Chlorella sorokiniana* KR904895, *Chlorella* sp. KM985404, *Scenedesmus obliquus* AB037088, *Scenedesmus* sp. KM985413, *Scenedesmus dimorphus* FR865725, *Scenedesmus* sp. KC859922, *Scenedesmus* sp. KF537681, *Desmodesmus armatus* AB917138, *Desmodesmus* sp. AB917097, *Desmodesmus* sp. AB917128, *Tetranephris brasiliensis* KF574392, *Monoraphidium* sp. KT279485 and *Coelastrum* sp. KT279487. The phylogenetic relationship of these strains is shown in Supplementary Figure 2. While *Desmodesmus* and *Chlorella* ssp. grouped closely

together, it is interesting to note that *Scenedesmus* sp. clustered to two distinct groups, demonstrating the high diversity within the *Scenedesmus* genus.

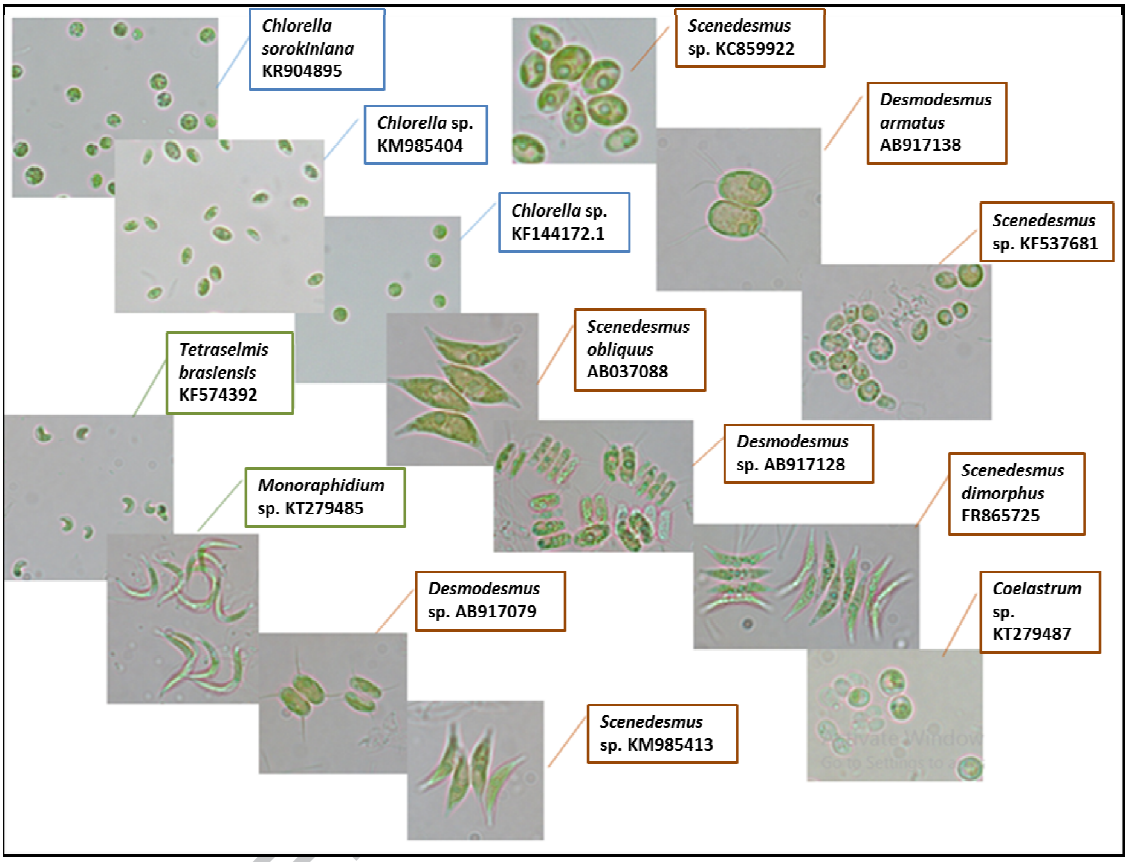

Figure 2. Images of distinct pure microalgal strains from freshwater samples.

Photographs of the mixed microalgal communities after initial growth and sequential supplementation with 1% CO_2, 5% CO_2 and air under laboratory conditions are shown in Supplementary Figure 3. Freshwater samples showed a mixed microbial community with many green microalgae (Chlorophyceae), most notably *Desmodesmus* sp., *Scenedesmus* sp. and *Chlamydomonas* sp., but also Trebouxiophycaea and *Ochromonas*, which belongs to the class of Chrysophyceae. The brackish/marine sample was dominated by *Skeletonema* sp. Microalgal community profiling by 18S rDNA amplicon sequencing of the combined lake cultures confirmed a high biodiversity (Figure 3; Laboratory samples). The most common microalgal taxa in lake samples belonged to the classes Chlorophyceae (52%) and Trebouxiophycaea (16%), as well as the genuses, *Ochromonas* (17%), *Desmodesmus* (10%)

and the species *Pseudopediastrum boryanum* (3%). Except for the latter these were also identified morphologically (Supplementary Figure 3). This culture also contained minute amounts of *Chlorococcum* sp. (0.2%), *Characiopodium* sp. (0.2%), *Streptophyta* (0.1%) and others (Figure 3; Supplementary Table 1). The two most common taxa in creek samples were Trebouxiophycaea (61%) and *Chlamydomonas fasciata* (37%) with small amounts of unspecified Chlorophycaea (1%) and *Poterioochromonas* sp. (0.4%).

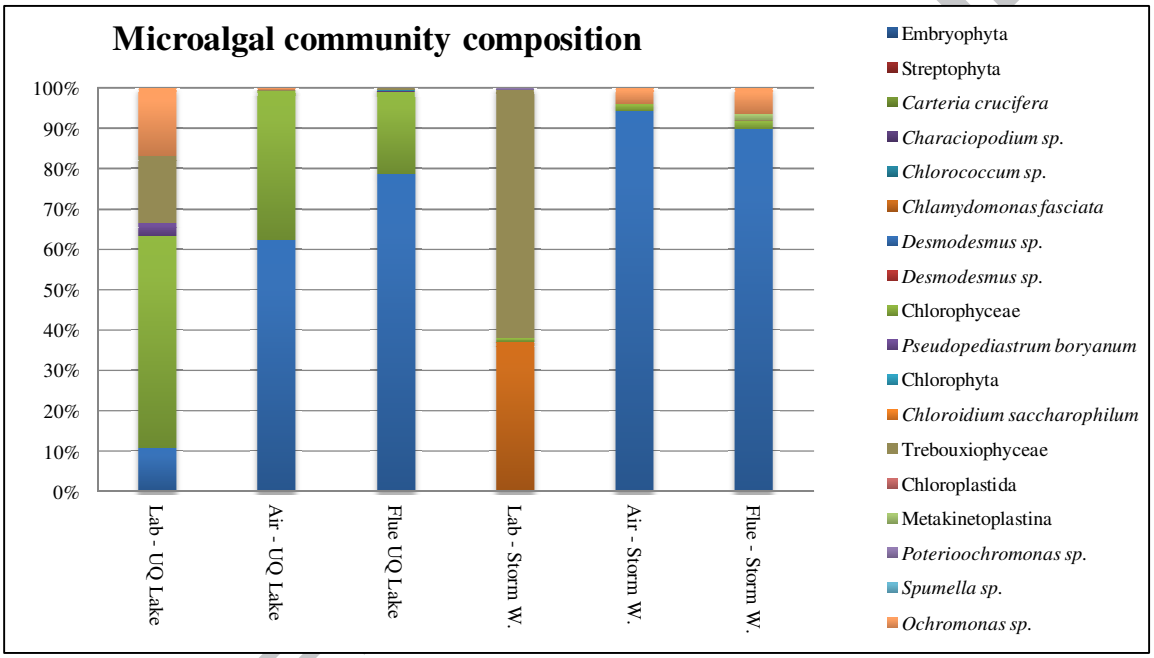

Figure 3. 18S rDNA sequencing of microalgal communities from freshwater cultures during the flue gas adaptation experiment. Shown are percentages of taxa present in cultures originating from a freshwater lake and a stormwater creek exposed to 5% CO_2 supplementation in the laboratory (Lab) at the start of the flue gas adaptation experiment, as well as the same cultures several months later after grown in photobioreactors under outdoor conditions with (Flue) or without (Air) exposure to 30% flue gas from a coal-fired plant. Abundant taxa (>1%) are shown in bold. The complete data are shown in Supplementary Table 1.

A high Shannon Diversity Index of 1.69 and a low Simpson's Index of 0.34 was calculated for lake water samples after initial adaptation to CO_2 under laboratory conditions, resulting in the highest Evenness of Species value (J = 0.41) for all cultures tested (Supplementary Figure 4). On the other hand, the storm water creek culture at the start of the experiment showed a

lower Shannon Index of 1.03 and a Simpson's Index of 0.52, resulting in an Evenness of Species value of 0.25. Figure 3, Supplementary Table 1 and the Shannon Diversity Index (Supplementary Figure 4) show that the diversity decreased as cultures adapted to outdoor conditions. This was occurring in both cases, with or without flue gas supplementation, suggesting that outdoor conditions, rather than flue gas exposure provided a higher selection pressure.

3.2. Growth and adaptation of microalgal cultures to flue gas

Cultures were subjected to cycles of growth phases and nutrient depletion with sequential increases in CO_2 supplementation. Each cycle lasted between 6 to 9 days. This served two purposes: the gradual adaptation of the cultures to elevated CO_2 levels and the ability to measure valuable algal products, such as lipids, that are expected to increase after nutrient depletion. After the initial adaptation to 1% CO_2 supplementation under laboratory conditions, cultures were subjected to 5% CO_2 supplementation and then air for comparison. Figure 4 displays productivities of these cultures under laboratory conditions and various exposures to bottled CO_2. Supplementary Figure 5 shows growth curves, nitrate and phosphate consumption as well as pH values for each experiment.

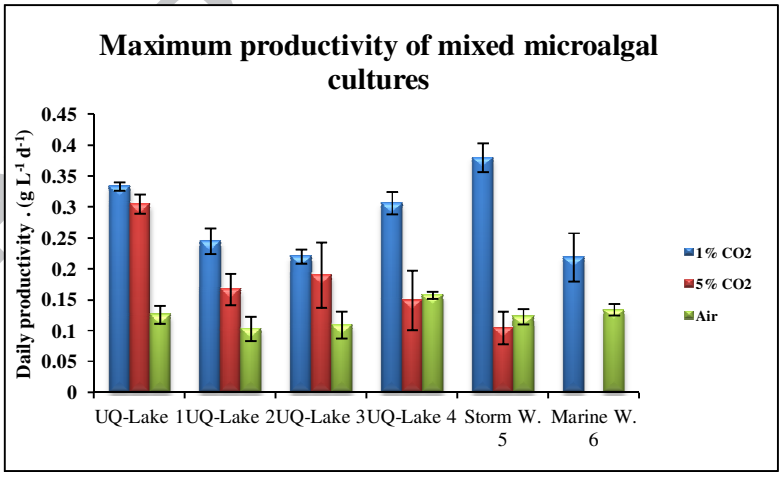

Figure 4. Maximum daily productivities (P_{max}) of mixed microalgal cultures to sequential pure CO_2 supplementation regimes. Shown are average values and SDs from three separately grown cultures under laboratory conditions, including four samples from a freshwater lake (UQ-Lake), one sample from storm water creek (Storm W.) and one sample from a brackish, tidal river (Marine W.).

For all cultures, 1% CO_2 supplementation had a significant (P<0.05) positive effect on productivity. However, supplementation with 5% CO_2 only had a significant positive effect on freshwater lake culture #1, a culture that was dominated by Trebouxiophycaea, *Desmodesmus* sp. and *Ochromonas* sp. (Supplementary Figure 3). The other cultures displayed no significant difference to when exposed to air only and the brackish/marine culture did not survive 5% CO_2 supplementation, presumably because of the strong decrease in pH. However, it was hypothesized that further adaptation, rather than selection, may first be required.

After the initial adaptation to elevated CO_2 levels under laboratory conditions, cultures were exposed to outdoor conditions in PBRs (three PBRs per treatment) with and without industrial flue gas supplementation. To maximize the gene pool and to reduce the number of required PBRs, the four lake-derived cultures were combined. Raw flue gas was collected from the smoke stack of a 4 MW coal-fired boiler by a sampling port which was then transferred to an air compressor where it was mixed with air to make up the required carbon dioxide concentration. Initial flue gas exposure was 10% (1.1% CO_2) which was increased after two weeks to 30% (3.3% CO_2). CO_2 measurements of ten times diluted flue gas (10%) before bubbling and after passing the algal cultures showed that 12.3-14.9% of the flue-derived CO_2 was absorbed after 10 min. Figure 5 summarizes daily productivities of cultures under nutrient replete and deplete conditions during the first 10% (1.1% CO_2) and the second 30% (3.3% CO_2) adaptation phase. As expected, cultures displayed higher productivities during nutrient replete conditions. However, there was no significant difference inferred from the supplementation of CO_2-rich flue gas. This is most likely because, unlike the laboratory-grown cultures, aeration of the PBRs was strong enough so that even in control (air-bubbled) cultures, CO_2 was not growth-limiting. The exposure to 10% (1.1% CO_2) and 30% (3.3% CO_2) did not markedly lead to a pH drop which were at 7.5 and 7, respectively, at the start of the experiment (Supplementary Figure 5). A comparison of productivities of laboratory-grown (Figure 4) and outdoor-grown cultures (Figure 5) during the nutrient replete phase shows similar or lower productivities under air-bubbling regime, suggesting that outdoor-grown cultures may be growth-limited by other factors, such as suboptimal lighting conditions.

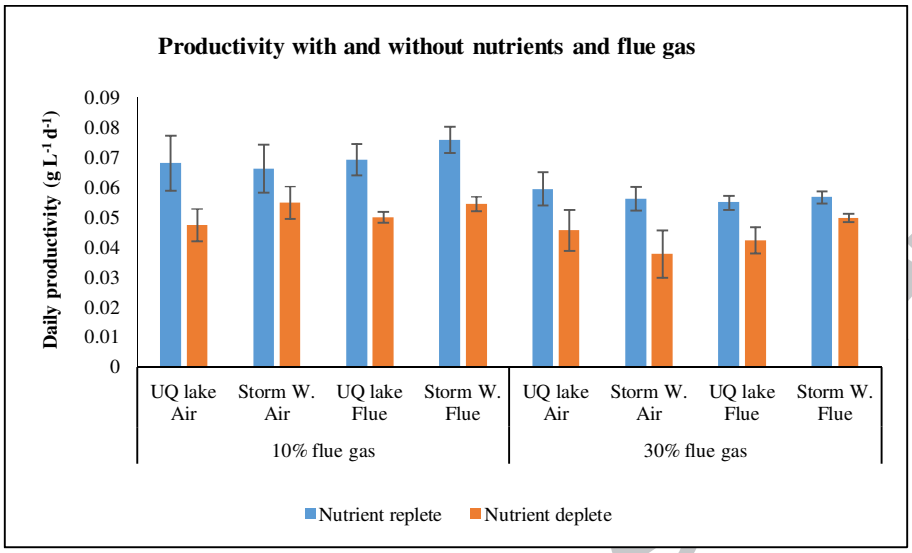

Figure 5. Daily productivities of mixed microalgal cultures to various flue gas supplementation regimes under nutrient replete and nutrient deplete conditions. Shown are average values and SDs from three separately grown cultures for each culture, including a preadapted combined freshwater lake (UQ-Lake) culture and a stormwater creek (Storm W.) culture.

Following the adaptation of microalgal consortia to flue gas in PBRs, the twelve cultures (three cultures, each, for lake and creek samples, with and without flue gas supplementation) were again subjected to 18S rDNA amplicon sequencing for diversity analysis. As shown in Figure 3, in all cases this led to a strong dominance of *Desmodesmus* sp., ranging from 62% to 94%. By comparison, *Desmodesmos* sp. contributed only a minor proportion (10% and only 0.008% for lake and storm water creek cultures, respectively) prior to outdoor adaptation (Figure 3; Supplementary Figure 4). As this dominance occurred in both, flue gas-supplemented as well as control air-bubbled cultures, this cannot be linked to the exposure to flue gas, but possibly has more to do with the conditions imposed by outdoor-grown PBR cultivation under vigorous bubbling. However, as this genus also dominated in high flue gas exposure conditions, it appeared to be not only vigorous but also highly adaptable and a rapid CO_2-to-biomass converter. A ten-fold higher abundance for flue gas-exposed cultures was found for *Pseudopediastrum boryanum* (0.029% to 0.279%) in lake cultures, while a two-fold higher abundance was observed for *Ochromonas* sp. in storm water creek cultures.

3.3. Adaptation of mixed microalgal consortia to 100% unfiltered flue gas

To test whether microalgal consortia can be further adapted to higher flue gas exposure, cultures adapted to growth in 30% (3.3% CO_2) flue gas, were exposed to 50% (5.5% CO_2) flue gas supplementation. However, cultures did not display any growth and two replicate cultures originating from stormwater creek (F2a and F2b) even died after 4-8 days as the pH declined to 3.6-4.8. Exposure to 100% flue gas even led to a pH decline to 2.3. Therefore, it was tested whether counterbalancing the strong effect of acidification by the NO_x and SO_x in flue gas by buffering the growth medium, may enable culture tolerance and further adaptation to higher flue gas concentrations.

In preliminary experiments with combined cultures following to 30% flue gas adaptation, HEPES, Tris and phosphate buffers with different concentrations were first tested in the laboratory with pure CO_2 at a flow rate 5 L min^{-1}. Phosphate buffer (50 mM) was chosen as the most suitable buffer as phosphate addition is required in any case for algae cultivation, with the difference that simply higher concentrations need to be maintained. The addition of higher phosphate concentrations had no detrimental effects on algae productivities under laboratory conditions without CO_2 supplementation (Figure 6). When exposed to supplementation with 10% pure CO_2, productivities increased as expected, but buffering did not further increase productivity, probably because the pH drop with pure CO_2 addition was not critically anymore to this mixed culture that had already previously been successfully adapted to 30% flue gas supplementation. In fact, the addition of buffer had slightly negative effects to productivity as the salinity of the culture increased by approx. 20 ppt.

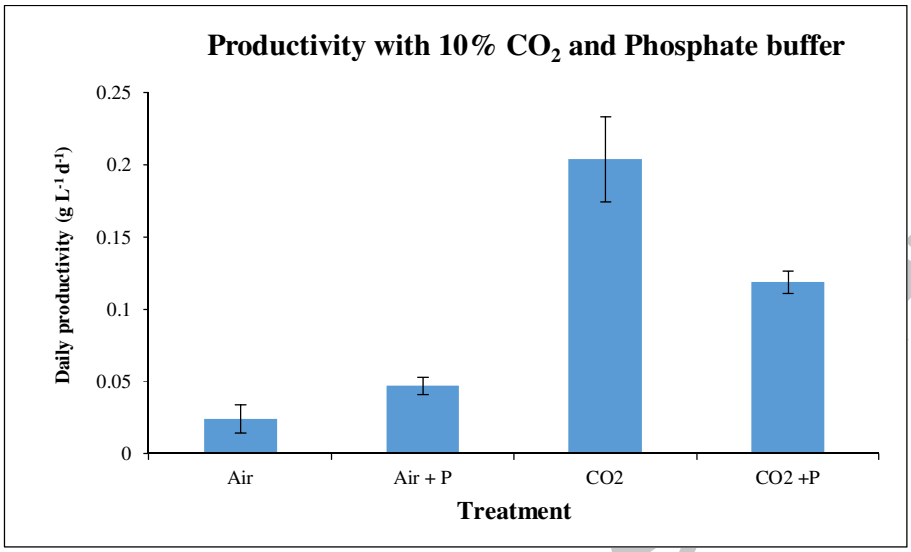

Figure 6. Productivity of flue gas-adapted mixed microalgal cultures with pure 10% CO_2 supplementation in the presence or absence of 50 mM phosphate buffer. Shown are average values ±SDs from three separately grown cultures under laboratory conditions.

Based on these results, high flue gas adaptation experiments in the presence and absence of phosphate buffer were carried out. To provide the best genetic material for cultivation and adaptation of microalgal communities to high level industrial flue gas supplementation, microalgal communities from both sources (UQ Lake and Storm W.) were first combined and then equally distributed into twelve PBRs at an inoculation density of 0.3 g L^{-1}. Again, three replicate experiments were carried out for each treatment: with and without the supplementation of high concentration flue gas and with and without the addition of 50 mM phosphate buffer. Flue gas supplementation to PBRs was then sequentially increased to 50% (5.5% CO_2), and then to 100% (11% CO_2) over a period of 4 weeks. CO_2 measurements before bubbling and after passing the buffered algal cultures showed that 7.6% of the flue-derived CO_2 was absorbed after 10 min. Figure 7A shows that an increase to 50% flue gas could still be tolerated, even in the absence of buffer. However, it also shows that the addition of flue gas did not lead to increased productivities, most likely because CO_2 was not a growth-limiting factor under these high rate bubbling conditions. Further flue gas supplementation to 100% had dramatic effects on all replicates that died rapidly without the presence of phosphate buffer which lead to a pH decrease of 2.4-4.5 (Figure 7B). The presence of phosphate buffer stabilized the pH to 5.7-6.5 during flue gas supplementation,

while the pH of cultures without flue gas was 9.0-9.5 irrespective of buffering. However, dry weight yields collected from buffered, flue gas-supplemented PBRs were still markedly lower than for cultures whose CO_2 supply was sourced from aeration only, suggesting that the low pH (5.7-6.5) or other flue gas substituents provide suboptimal conditions. Further cultivation under these low pH/high salinity conditions under more carbon-limiting conditions may lead to further adaptation to the detrimental effects of flue gas and higher productivities. Nevertheless, the data from this study collectively demonstrate that microalgal growth with 100% unfiltered flue gas from a coal-fired power plant is possible after adaptation of mixed microalgal consortia over several months. The composition of these cultures was dominated by *Desmodesmos* sp., but also *Chlorella* sp., *Scenedesmus* sp. and filamentous cyanobacteria (Supplementary Figure 6).

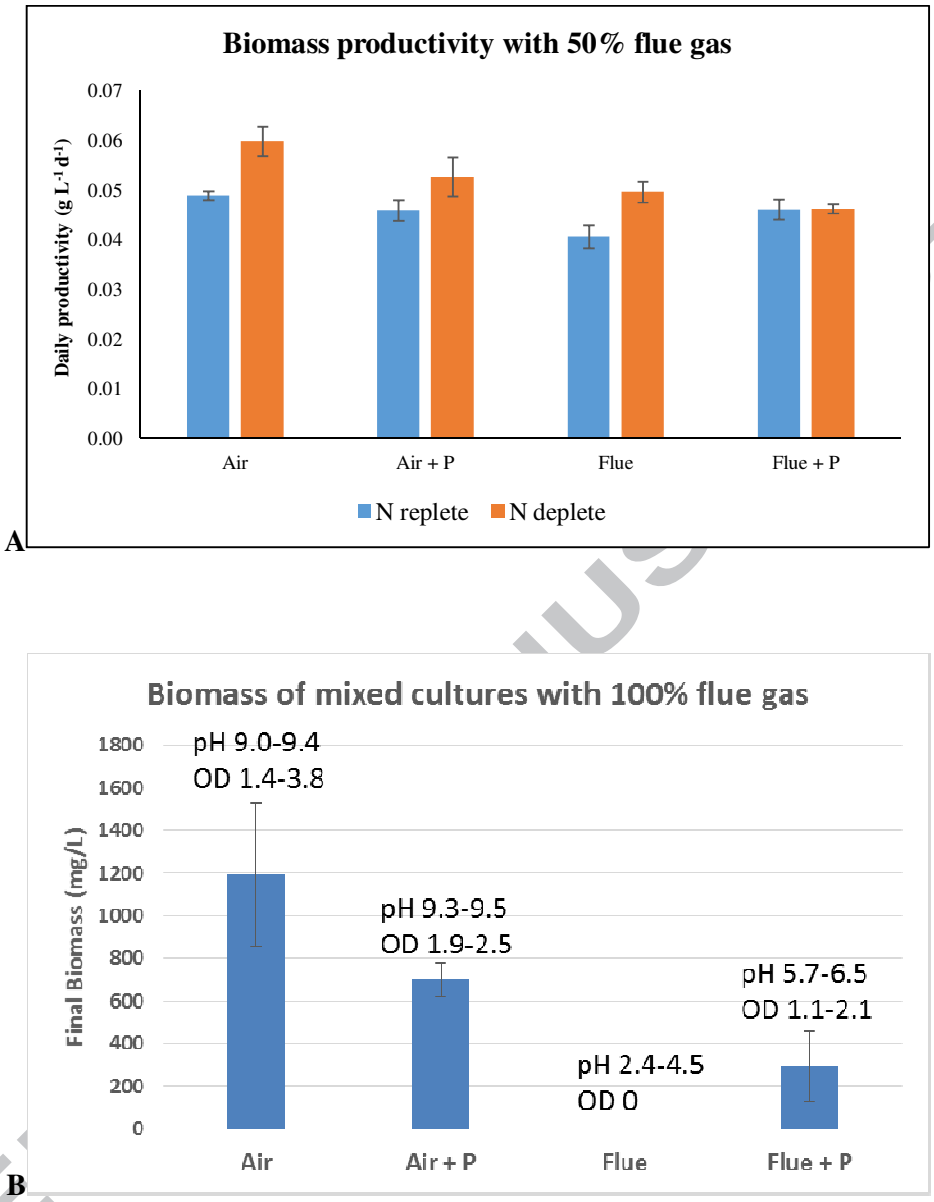

Figure 7. Biomass productivity and biomass at completion of flue gas adaptation experiment. A. Overall biomass productivities from 50% flue gas supplementation during the nitrogen replete or deplete conditions. **B.** Final biomass, OD 450 nm and pH conditions after supplementation with 100% flue gas for 2 weeks. Shown are average values ±SDs from three separately grown PBRs with or without flue gas supplementation in the presence or absence of phosphate buffer compared to air-only control cultures.

3.4. Value-add processing of algal biomass following flue gas cultivation

Cultures in PBRs underwent phases of exponential growth under nutrient replete conditions followed by phases of nutrient deplete conditions in an effort to induce lipid biosynthesis. FAME contents and productivities of microalgal cultures supplemented with coal-fired flue gas ranged from (7.4% DW; 3.4 mg L^{-1} d^{-1}) to (28.0% DW; 14.0 mg L^{-1} d^{-1}), respectively (Table 1).

Table 1. Fatty acid methyl ester contents from mixed microalgae consortia (lake and stormwater creek or combined) grown in the presence (F) and absence (A) of various flue gas supplementation. All samples were harvested during the nutrient depletion phase. Mixed cultures with 50% flue gas supplementation included phosphate buffering (P).

Flue gas	Samples	Total FAME Content (% DW ± SE)	Biomass productivity (mg L^{-1} d^{-1}) under nutrient depletion	FAME Productivity (mg L^{-1} d^{-1})
10%	UQ-Lake (A)	16.56 ±1.23% a	47.458	7.86
	UQ-Lake (F)	28.03 ±0.79% b	50.042	14.03
	Storm W. (A)	24.82 ±0.87% c	54.875	13.62
	Storm W. (F)	24.95 ±0.75% c	54.5	13.60
30%	UQ-Lake (A)	12.77 ±0.28% a	45.689	5.84
	UQ-Lake (F)	16.86 ±0.46% b	42.444	7.16
	Storm W. (A)	14.61 ±3.22% ab	37.733	5.51
	Storm W. (F)	21.00 ±2.45% c	49.867	10.47
50%	Mixed C. (A)	10.38 ±1.10% a	59.75	6.20
	Mixed C. (F)	10.00 ±1.64% a	49.542	4.95
	Mixed C. + P (A)	7.02 ±0.30% b	52.583	3.69
	Mixed C. + P (F)	7.44 ±0.24% b	46.167	3.44

Shown are mean FAME values ± SEs from three separately-grown PBRs. Different small letters indicate statistically significant differences (Tukey's test $P<0.05$) within a flue gas supplementation regime.

The main fatty acid components were C16:0, C16:1, C16:2, C16:3, C16:4, C18:0, C18:1, C18:2, C18:3 and C18:4 (Supplementary Table 2). Fatty acid profiles observed from the mixed cultures varied under different CO_2 concentrations and the percentage of SFA

increased as flue gas supplementation increased. The combined cultures (Mixed C.) under 50% flue gas produced 31.5-36.3% SFA of total FAME, while this fraction only made up 23.3-26.3% in cultures with 10% flue gas supplementation (Table 1). This was mostly attributed to C16:0 contents that were higher (30.6-32.6% C16:0) with high (50%) flue gas supplementation, compared to 10% flue gas addition (21.5-23.7% C16:0).

Total soluble protein of flue gas-grown microalgae from the present study varied from 25.9 to 39.3% DW but decreased markedly by as much as 59% during nitrogen deplete conditions (Supplementary Table 3). Pure strains that were isolated from the cultures (Figure 2) displayed varying protein contents, but two strains with homology to *Desmodesmos* sp. AB917097 and *Scenedesmus dimorphus* FR865725 stood out with 62.9% and 51.3% (w/w) protein, respectively (Supplementary Figure 7). Future research may focus on the ability of adapted *Desmodesmus* sp. to not only grow under elevated flue gas supplementation regimes, but also its high protein content.

4. Discussion

To the authors' knowledge, the present study is the first that was able to demonstrate that microalgal cultures can be adapted to tolerate high rate coal-fired flue gas supplementation up to 100%. The main challenge was the steep drop in pH in the medium caused by the presence of SO_x and NO_x. This required a slow adaptation phase of mixed microalgal populations and the addition of phosphate buffer at high (>50%) flue gas supplementation. This study was not designed to capture 100% of the CO_2 emissions or to fully optimize the process of microalgal flue gas capture, but focused instead on the hypothesis that mixed microalgal consortia can be adapted over time to grow under these adverse conditions. This proof-of-concept has been established in the present study, however growth optimization, scaling up and possibly further adaptation will be required to establish this as an industrial process.

One of the main purposes of this project was to investigate if and how a mixed microalgal community can adapt to increasing supplementation of unfiltered coal flue gas. The process occurring after inoculation of a culture to changed conditions should be considered selection, rather than adaptation and depended on the provision of a high incoculum (0.2-0.3 g L^{-1}), suggesting that a high proportion of microalgal cells died. On the other hand, gradual adaptation of microalgal consortia to high CO_2/flue gas supplementation regimes was also required over 2 weekly intervals, as initial growth (even with 10% pure CO_2 was not

possible). This is consistent with previous studies that showed that above 5% CO_2 concentration, microalgal growth and CO_2 biofixation can be inhibitory (Chiu *et.al.* 2008; de Morais and Costa, 2007b; Cheah *et al.* 2015). An observable trend that culminated on the final day of the experiment was that over time, the community assemblage showed how a few species dominate (mainly *Desmodesmus* sp. and cyanobacteria). The dynamics of the population depended mainly on two factors (1) the ability to tolerate low pH and other adverse effects from coal flue gas, and (2) the ability to rapidly produce biomass. The latter is directly related to CO_2 uptake.

Microalgal lipids are a center of attention because they can potentially be used for biodiesel production and some fatty acids are of high value (e.g. omega-3 fatty acids; Vauhkonen *et al.* 2009; Adarme-Vega *et al.* 2014). High rates of aeration reportedly led to comparatively more C16-C18 fatty acids in some microalgal species (Knothe, 2009). This carries important implications for long term and stable microalgal culture processes, specifically with respect to industrial exhaust flue gases. A high level of saturated fatty acid tends to enhance the stability of biodiesel as unsaturated fatty acids have poor oxidative stability (Demirbas, 2008). Algal FAME contents were reported 0.961 g L^{-1} and 0.792 g L^{-1} from hot-stove (at a 1/4 dilution) and power plant (at a 1/2 dilution) exhaust gases respectively under autotrophic conditions (Kao *et al.* 2014). However, a decrease in the lipid content of algal biomass had previously been associated with higher CO_2 content in gas (Yoo *et al.* 2010), a trend that was also visible in the present study. This could be caused by the continuous nitrogen supply to the cultures in the form of NO_x during flue gas supplementation (although nitrate levels were not measurably higher in the presence of flue gas; Supplementary Figure 5). In addition, as the adaptation of the population resulted in a higher proportion of cyanobacteria (Supplementary Figure 6), the higher CO_2 levels did not necessarily result in higher lipid contents. Despite this the algal biomass can still be used for many other purposes, for example hydrothermal liquefaction to produce biocrude, anaerobic digestion to produce biogas, or biochar to enable carbon capture and sequestration. The culture itself is also valuable as these adapted strains can be used in the future when mitigating coal flue gas emissions. A simple inoculation with this culture (similar to sludge from anaerobic digesters) may allow immediate use under high percentage flue gas supplementation regimes.

Kumar *et al.* 2014 used a reactor-in-series strategy for CO_2 fixation from industrial flue gas and observed major reductions in CO_2 (4.1%, v/v) along with other hydrocarbons in serially

connected photobioreactors. In another study, De Morais and Costa (2007a) used three-stage tubular PBRs to assess growth of *Arthrospira* (*Spirulina*) sp. and *Scenedesmus obliquus* for CO_2 fixation under different CO_2 concentrations and observed an increase in biomass productivity as well as CO_2 fixation towards the downstream reactor. The third PBR, at the end of the serially-connected PBRs, showed lower biomass density because of a lower gas flow rate and possibly lower CO_2 concentrations (Lee *et al.* 2000; De Morais and Costa 2007a). Carbon dioxide removal efficiency by microalgae can range from 1.5% to 92% (most of studies showing the average removal of 50%) (Cheah *et.al.* 2015; De Morais & Costa 2007b).

Lizzul *et al.* (2014) observed an increase in biomass yield and lipid production by addition of CO_2, but Chiu *et.al.* (2008) reported a significant reduction in microalgal cell growth while treating *Chlorella* sp. culture with high concentrations of CO_2 gas. *Chlorella* sp. has exhibited satisfactory growth with CO_2 concentrations ranging from 1% to 18% v/v (Hulatt and Thomas, 2011) but it has also been shown to grow at extreme conditions of up to 50% CO_2 concentration (Maeda *et.al.* 1995). Flue gas with 10-15% CO_2 concentration can also be inhibitory to algal growth simply based on the pH drop inferred by high CO_2 supply (Hauck *et.al.* 1996). While laboratory experiments with mixed cultures in the present study clearly demonstrated higher biomass productivities with pure CO_2 supplementation (Figures 6), the supplementation with flue gas showed lower productivities compared to air-only control PBRs (Figure 7). Therefore, there is a necessity to further adapt cells to these adverse conditions (including the low pH) and manage the negative effects (e.g. by buffering and/or flue gas filtering). Measurements in this study showed that only 7.6-14.9% of the flue-derived CO_2 was absorbed after 10 min. To ensure that all CO_2 from flue gas is taken up, serial PBRs can be constructed (Kumar *et.al.* 2014; De Morais and Costa, 2007a).

Apart from lowering the pH, NO_x and SO_x in flue gas also directly inhibits microalgal growth (Hauck *et.al.* 1996). In the present study, the coal-fired flue gas contained 11.24% CO_2, 423.9 mg/Nm^3 (209.2 ppm) NO_x and 781.8 mg/Nm^3 (277.2 ppm) SO_x. Dissolved NO from NO_x is considered as an alternate nitrogen source for cell growth and should not have any significant negative impact to microalgal growth. Conversely, SO_2 concentration in flue gas above 60 ppm is considered inappropriate to be used for algae cultivation (Lam *et al.* 2012). The presence of 100-250 ppm SO_2 reduces the pH to 2.5-3.5 due to formation of sulphonic and sulphuric acid in the medium (Lam *et al.* 2012; Zhao and Su, 2014). In the present study, the

addition of 100% flue gas (11.24% CO_2) led to a pH reduction to 2.4-4.5 (Figure 7B). Reduced pH decreases the activity of extracellular carbonic anhydrase and inhibits cell growth (Tang *et al.* 2011) and many microalgal species are even unable to withstand the acidic conditions from carbonic acid formed by CO_2 dissolution in medium (Lam and Lee, 2011). High-density cultures have shown better ability to tolerate the harsh conditions by toxic flue gas elements (Chiu *et al.* 2008). It has been reported that regulating pH and increasing the inoculating cell density are effective ways to prevent growth inhibition exerted by flue gas (Lee *et al.* 2002). This was confirmed by the present study, which found that a low cell density of 0.1 g DW L^{-1} in the inoculum to start cultures in PBRs supplemented with 50% (5.5% CO_2) flue gas resulted in culture death, while a higher inoculation density of 0.2-0.3 g DW L^{-1} was suitable for continued growth and culture adaptation. Most likely, a large proportion of the inoculated algae died on the first day. Nevertheless, the growth curves, even at the higher flue gas supplementation of 50% (5.5. CO_2) showed clear growth (Supplementary Figure 5). The OD increased approx. 10-fold in 2 weeks. Regulating the pH by supplying 50 mM phosphate buffer in the medium clearly enabled the cultures to keep growing (Figure 7), but it negated the positive effect found for growth with lower CO_2 supplementation. Therefore, if flue gas CO_2 is to be removed completely by using serial closed or open PBRs, each PBR may house a culture that is adapted to a different degree to the presence of flue gas.

5. Conclusion

CO_2 emissions from coal-fired power plants continue to significantly contribute to global greenhouse gas emissions. This study demonstrates proof-of-concept that mixed microalgal communities can be slowly adapted to grow in 100% unfiltered flue gas from coal combustion. Resilient *Desmodesmus* spp. were identified as dominant microalgae under these suboptimal conditions that also required buffering. To achieve significant CO_2 uptake from coal-fired flue gas, upscaling and serial passages of flue gas should be implemented over several open or closed PBRs. To achieve net carbon emission reductions, the obtained microalgal biomass can be used for biofuel production or carbon sequestration (e.g. biochar).

Acknowledgements

We wish to thank Alex Hood from Australian Country Choice for his kind cooperation and the installation of the flue gas supply to the microalgal PBRs and are grateful to the Australian Research Council (LP0990558) and the Higher Education Commission Pakistan for financial support

References

1. Adarme-Vega, T. C., Thomas-Hall, S. R., Schenk, P. M. (2014) Towards sustainable sources for omega-3 fatty acids production. *Current Opinion in Biotechnology* 28, 14-18.
2. APHA (American Public Health Association) (1998), Standard methods for the examination of water and waste water. APHA. AWWA. WPFC, 16 Ed. New York.
3. Betts, R.A., Jones, C.D., Knight, J.R., Keeling, R.F. and Kennedy, J.J., 2016. El Nino and a record CO_2 rise. *Nature Climate Change*, 6, 806–810.
4. Binaghi, I., Borghi, A. D., Lodi, Converti, A. and Borghi, M. D. 2003. Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis*. *Journal of Process Biochemistry*, 38(9), 1341-1346.
5. Chaturvedi, R. K., Sharma, K. P., Sharma, K., Bhardwaj, S. M. and Sharma, S., (1999), Plankton community of polluted water around Sanganer, Jaipur, *Journal of Environmental Pollution*, 61, pp 77-84.
6. Cheah, W. Y., Show, P. L., Chang, J. S., Ling, T. C., & Juan, J. C. (2015). Biosequestration of atmospheric CO_2 and flue gas-containing CO_2 by microalgae. *Bioresource Technology*, 184, 190-201.
7. Chiu, S. Y., Kao, C. Y., Chen, C. H., Kuan, T. C., Ong, S. C., & Lin, C. S. (2008). Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. *Bioresource Technology*, 99(9), 3389-3396.
8. De Morais, M. G., & Costa, J. A. V. (2007a). Biofixation of carbon dioxide by Spirulina sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *Journal of Biotechnology*, 129(3), 439-445.
9. De Morais, M. G., & Costa, J. A. V. (2007b). Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Conversion and Management*, 48(7), 2169-2173.
10. Demirbaş, A. (2008). Production of biodiesel from algae oils. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 31(2), 163-168.

11. Duong, V. T., Li, Y., Nowak, E., Schenk, P. M. (2012) Microalgae isolation and selection for prospective biodiesel production. *Energies* 5, 1835-1849.
12. Guillard, R. R. L. & Ryther, J. H. (1962) Studies of marine planktonic diatoms: I. *Cyclotella Nana* Hustedt, and *Detonula confervacea* (CLEVE) Gran. *Canadian Journal of Microbiology*, 8, 229-239.
13. Halim, R., Danquah, M.K. and Webley, P.A., (2012). Extraction of oil from microalgae for biodiesel production: A review. *Biotechnology Advances*, 30(3), 709-732.
14. Hauck, J. T., Scierka, S. J., & Perry, M. B. (1996). Effects of simulated flue gas on growth of microalgae. Preprints of Papers, *American Chemical Society, Division of Fuel Chemistry*, 41, 1391-1396.
15. Hsueh, H. T., Chu, H. and Yu, S. T. (2007). A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot springs and marine algae. *Journal of Chemosphere*, 66, 878-886.
16. Hulatt, C. J., & Thomas, D. N. (2011). Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. *Bioresource Technology*, 102(10), 5775-5787.
17. Kadam, K.L. 1997. Power-Plant Flue Gas as a Source of CO_2 for Microalgae Cultivation: Economic Impact of Different Process Options. *Journal of Energy Conversion & Management*, (38): S505-S510.
18. IEA (International Energy Agency) (2015). CO_2 emissions from fuel combustion, Highlights, IEA Publications, Paris.
19. Kao, C. Y., Chen, T. Y., Chang, Y. B., Chiu, T. W., Lin, H. Y., Chen, C. D., ... & Lin, C. S. (2014). Utilization of carbon dioxide in industrial flue gases for the cultivation of microalga *Chlorella* sp. *Bioresource Technology*, 166, 485-493.
20. Knothe, G. (2009). Improving biodiesel fuel properties by modifying fatty ester composition. *Energy & Environmental Science*, 2(7), 759-766.
21. Knothe, G. (2011). A technical evaluation of biodiesel from vegetable oils vs. algae. Will algae-derived biodiesel perform? *Green Chemistry* 13 (11), 3048–3065.
22. Kumar, K., Banerjee, D., & Das, D. (2014). Carbon dioxide sequestration from industrial flue gas by *Chlorella sorokiniana*. *Bioresource Technology*, 152, 225-233.
23. Lam, M. K., & Lee, K. T. (2011). Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win–win strategies toward better environmental protection. *Biotechnology Advances*, 29(1), 124-141.

24. Lam, M. K., Lee, K. T., & Mohamed, A. R. (2012). Current status and challenges on microalgae-based carbon capture. *International Journal of Greenhouse Gas Control*, 10, 456-469.
25. Lee, J. N., Lee, J. S., Shin, C. S., Park, S. C., & Kim, S. W. (2000). Methods to enhance tolerances of *Chlorella* KR-1 to toxic compounds in flue gas. In Twenty-First Symposium on Biotechnology for Fuels and Chemicals (pp. 329-342). Humana Press.
26. Lee, J. S., Kim, D. K., Lee, J. P., Park, S. C., Koh, J. H., Cho, H. S., & Kim, S. W. (2002). Effects of SO_2 and NO on growth of *Chlorella* sp. KR-1. *Bioresource Technology*, 82(1), 1-4.
27. Lim, D. K., Garg, S., Timmins, M., Zhang, E. S., Thomas-Hall, S. R., Schuhmann, H. Li, Y. & Schenk, P. M. (2012) Isolation and evaluation of oil-producing microalgae from subtropical coastal and brackish waters. *PLoS ONE*, 7(7), e40751.
28. Lizzul, A. M., Hellier, P., Purton, S., Baganz, F., Ladommatos, N., & Campos, L. (2014). Combined remediation and lipid production using *Chlorella sorokiniana* grown on wastewater and exhaust gases. *Bioresource Technology*, 151, 12-18.
29. López, C. V. G., García, M. D. C. C., Fernández, F. G. A., Bustos, C. S., Chisti, Y., & Sevilla, J. M. F. (2010). Protein measurements of microalgal and cyanobacterial biomass. *Bioresource Technology*, 101(19), 7587-7591.
30. Macintyre, H.I., Cullen, J.J., and Andersen R.A. 2005. Algal Culturing Techniques (ed. by), Elsevier Acad. Press, Amsterdam, pp: 287-326.
31. Maeda, K., Owada, M., Kimura, N., Omata, K., & Karube, I. (1995). CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae. *Energy Conversion and Management*, 36(6), 717-720.
32. McGinn, P. J., Dickinson, K. E., Bhatti, S., Frigon, J. C., Guiot, S. R., & O'Leary, S. J. (2011). Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. *Photosynthesis Research*, 109(1-3), 231-247.
33. Moheimani, N.R. (2015). *Tetraselmis suecica* culture for CO_2 bioremediation of untreated flue gas from a coal-fired power station. *Journal of Applied Phycology* 28, 2139–2146.
34. Moreira, D. and Pires, J.C., (2016). Atmospheric CO_2 capture by algae: negative carbon dioxide emission path. *Bioresource Technology*, 215, 371-379.

35. Nichols, H. W. (1973) Growth media – freshwater. In Stein, J. (Ed.) Handbook of Phycological Methods, Culture Methods and Growth Measurements, Camb. Univ. Press pp. 7-24.
36. Pielou, E.C., (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, 13, pp 131-144.
37. Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425.
38. Shannon, C. E. and Weaver, W., (1949). The mathematical theory of communication. University of Ilinois Press, Urbana, pp. 125.
39. Simpson, E. H., (1949), Measurement of diversity, *Nature*, 163, pp. 688-689.
40. Stewart, C. and Hessami, M.A., (2005). A study of methods of carbon dioxide capture and sequestration—the sustainability of a photosynthetic bioreactor approach. *Journal of Energy Conversion & Management*, 46, 403-420.
41. Supekar, S.D. and Skerlos, S.J., (2015). Reassessing the efficiency penalty from carbon capture in coal-fired power plants. *Environmental Science & Technology*, 49(20), 12576-12584.
42. Tang, D., Han, W., Li, P., Miao, X., & Zhong, J. (2011). CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. *Bioresource Technology*, 102(3), 3071-3076.
43. Van Den Hende, S., Vervaeren, H., & Boon, N. (2012). Flue gas compounds and microalgae: (Bio-) chemical interactions leading to biotechnological opportunities. *Biotechnology Advances*, 30(6), 1405-1424.
44. Vauhkonen, V., Niemi, S., Hiltunen, E., Salminen, H. J., & Pasila, A. (2009). The first generation biodiesel: the effects of raw material on physical properties, oxidation stability and emissions. In Clean Electrical Power, 2009 International Conference on (pp. 117-123). IEEE.
45. Yoo, C., Jun, S. Y., Lee, J. Y., Ahn, C. Y., & Oh, H. M. (2010). Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresource Technology*, 101(1), S71-S74.
46. Zhao, B., & Su, Y. (2014). Process effect of microalgal-carbon dioxide fixation and biomass production: a review. *Renewable and Sustainable Energy Reviews*, 31, 121-132.

- Mixed microalgal consortia were adapted to grow in coal-fired flue gas
- Step-wise adaptation to increasing flue gas was applied over several months
- Phosphate buffering enabled growth in 100% unfiltered flue gas
- *Desmodesmus* spp. were among the most resilient and dominant microalgae