Materials and Methods - Survival of the Fattest – DOI: 10.1039/c3ee42912a

A non-sterile 1,5 L bioreactor (Applikon, Schiedam, The Netherlands) with a diameter of 11 cm and height of 17 cm was run in a sequenced batch mode for three months with cycles of 24 hour. Figure M&M-1 describes the operation cycle.

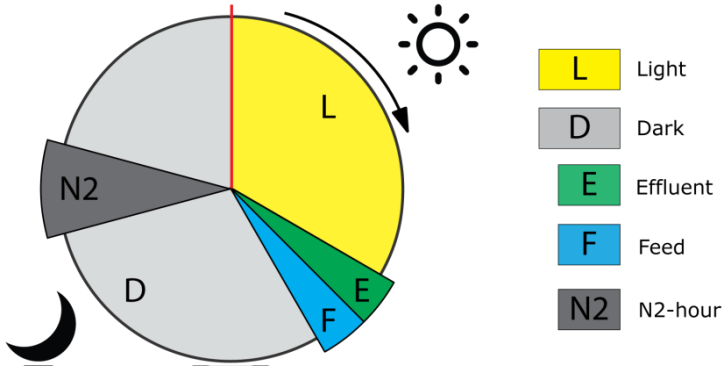

Figure M&M-1: overview of the operational cycle

During the N_2 period the reactor was sparged with N_2 gas to decrease protozoa growth. The reactor was run using the following operational parameters: volume 1320 mL, cycle length 24 h, light period 8 h, dark period 16 h, solid retention time 33.3 h, temperature 28 ºC, stirrer speed 200 rpm, gas flow reactor in and out 40 mL/min, gas recycle over reactor 1500 mL/ min, gas composition 5% CO_2 in air and 5% CO_2 in N_2 during N_2 period, average light intensity at inner reactor surface 650 µmol / m^2 s^{-1} provided by HPS lamps, pH setpoint 7.5, base 1 M $NaHCO_3$, acid 0.5 M HCl. All liquid and gas flows entering and leaving the system were not sterilised. The reactor was cleaned once a week in a non-sterile environment to remove any biofilm. Several samples for surface water were used as inoculum.

A Bio Controller ADI 1030 (Applikon, Schiedam, The Netherlands) controlled Masterflex pumps (Cole-Parmer, Vernon Hills, IL, USA) and mass flow controllers (Brooks Instruments, Ede, The Netherlands). The Bio Controller itself was controlled by a PC with MFCS_win software (Sartorius Stedim Systems, Goettingen, Germany)

A modified COMBO-medium was used (Kilham, Kreeger et al. 1998). The final nitrogen concentration was 20 mg N / L. To ensure N limitation all concentrations except for the N-source were multiplied by a factor twelve. The following modifications were made to the recipe: NH_4Cl instead of $NaNO_3$, no $NaHCO_3$, No KCl, no animal trace elements, no vitamins, addition of 10 mg / L allylthiourea to prevent nitrification. The N-source was dosed separately from the rest of the medium in peaks throughout the night.

pH and DOT were continuously measured by the Bio Controller ADI 1030. Offgas CO_2 and O_2 were analysed using a Rosemount NGA offgas analyser (Emerson, USA). NH_4 was determined spectrophotometrically using Dr. Lange LCK 403 NH_4 cuvette tests (Hach Lange, Dusseldorf, Germany).

Samples were taken at the transition from dark to light and from light to dark in steady-state after six weeks of cultivation. Reactors were assumed to be in steady state when offgas CO_2, O_2 and pH showed similar profiles during multiple subsequent cycles. Besides, NH_4 should be depleted before the end of the dark period. Three consecutive cycles were measured.

Total dry weight was determined by centrifuging and freezedrying a known amount of sample. Ash content was determined by burning freezedried biomass for 1 hour at 550 degrees.

Volatile Suspended Solids (VSS) was obtained by subtracting ash amount from the total dry weight.

Lipid extraction was done as described by (Johnson, Kleerebezem et al. 2009) Extracted lipids were analysed for the presence of PHB, myristic, palmitic, oleic, stearic, linoleic and linolenic acid by gas chromatography (model 6890N, Agilent, Lexington, MA, USA) equipped with a flame ionization detector on a HP Innowax column. Unidentified lipid peaks were not taken into account.

Polyglucose was measured by heating approx 5 mg freezedried biomass with 0.6 M HCl for three hours at 100 ºC. After centrifugation and filtration with a 0.45 μm pore size filter (PVDF Membrane, Millipore, Tullagreen, Ireland) the polyglucose concentration was determined by high performance liquid chromatography, using an Aminex HPX-87H column from Bio-Rad (Hercules, CA, USA) (t=60 ºC) coupled to an ultraviolet and a refractive index detector. The obtained lipid and polyglucose were multiplied with the total dry weight to yield total polyglucose and lipids. Biomass for the transition from dark to light was calculated as VSS minus polyglucose and lipids. Since it is highly likely that other storage compounds are also produced during the light period the biomass at the transition from light to dark was calculated in a different way. The biomass was assumed to be linearly related to the cell number. To calculate the biomass at the transition from light to dark the biomass at the transition from dark to light was multiplied by the increase in cell number during the light period.

Pictures were taken using a Leica DM500B light microscope (Leica Microsystems, Wetzlar, Germany) equipped with fluorescence filtercube A (excitation: UV, excitation filter: BP 340-380, dichromatic mirror: 400, suppression filter: LP 425). 1 µl BODIPY 505/515 (Invitrogen D3921, Life Technologies, Grand Island, USA) in DMSO (1 mg/ml) was used to colour lipids in 1 mL of algal cells. 10 µl Lugol's solution was used to colour starch in 1 mL of algal cells. Cell number and concentration were

determined by counting at least 300 algal cells using a counter chamber (W. Schreck, Hofheim, Germany).

The microbial composition was analysed using the PCR-DGGE technique. Genomic DNA was extracted using the Powersoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The subsequent extracted DNA was used for amplifying bacterial 16S RNA using the primer pair BAC341F with a GC clamp and BAC907RM (Bassin, Kleerebezem et al. 2012), eukaryotic 18S RNA using the primer pair EUK1F and EUK563R with a GC clamp (Diez, Pedros-Alio et al. 2001) and chloroplast 16S RNA from the eukaryotes using the primer pair CHL21F and CYA781RW without GC clamp. For DGGE we reamplified the CHL21F-CYA781rW fragment using the primer pair CYA359F with GC clamp and CYA781rW. The temperature programme was the same as described by (Bassin, Kleerebezem et al. 2012)

The 16S and 18S RNA gene amplicons were applied onto a 6% polyacrylamide gel with a denaturing gradient from 10 to 60% UF. (100% denaturant is a mixture of 5.6 M urea and 32% formamide). (Schäfer and Muyzer 2001) Individual bands were excised with a sterile razor and incubated overnight in 40 µL 10 mM tris solution water at 4 °C. Re-amplification was performed using the same primer pairs without GC clamp for 25 cycles under the same conditions as above. The purity of the PCR products were checked on an agarose gel. Subsequently, the PCR products were sequenced by Macrogen Inc. (Amsterdam, the Netherlands).

References

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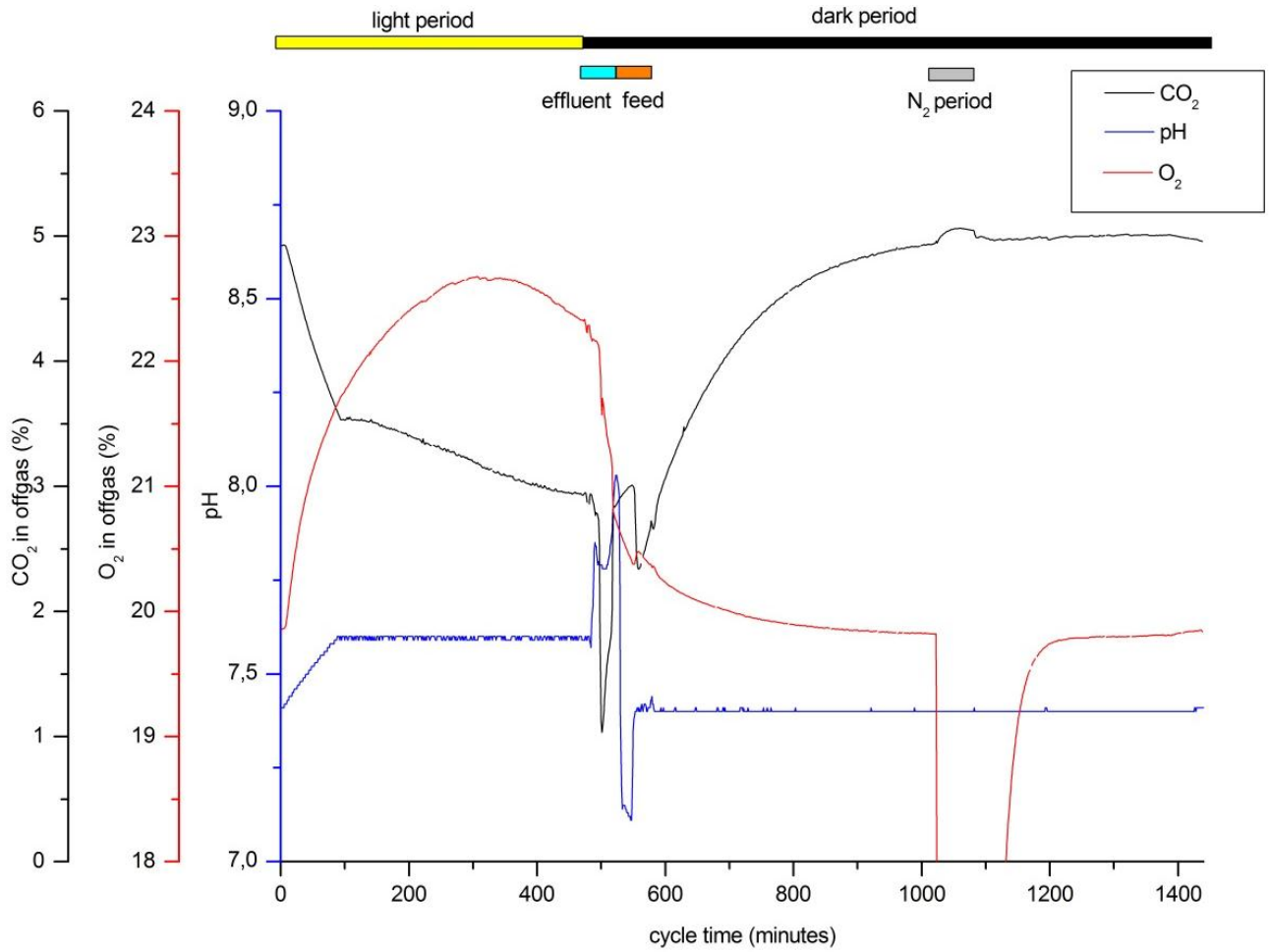
Supplementary Material - Survival of the Fattest – DOI: 10.1039/c3ee42912a



Supplementary Figure 1: typical pH, CO_2 and O_2 offgas profiles during a cycle.

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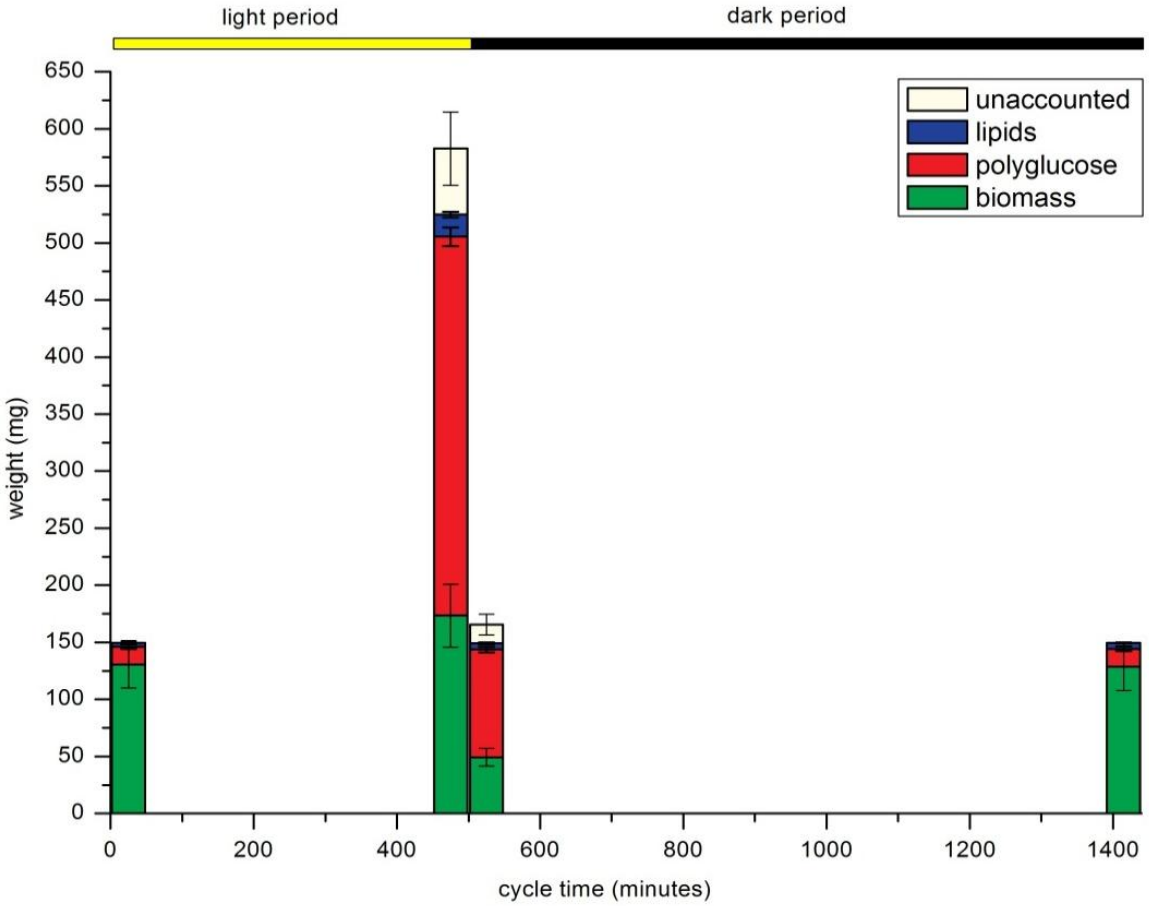

Supplementary Figure 2: biomass, lipid, polyglucose and unaccounted weight during three consecutive cycles after six weeks of cultivation. Error bars denote standard deviation. The sudden decrease at t=480 min is due to effluent and feed.

Supplementary Figure 2 has been used to construct Figure 1 in DOI: 10.1039/c3ee42912a