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A flexible microbial co-culture platform for simultaneous utilization of methane and carbon dioxide from gas feedstocks

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HIGHLIGHTS

- A co-cultivation technology that converts, CH_4 and CO_2, into microbial biomass.
- Robust bacterial growth on biogas and natural gas feedstocks.
- Continuous co-cultivation without air or O_2 feed to support CH_4 oxidation.
- A flexible co-culture technology constructed from genetically tractable bacteria.

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ABSTRACT

A new co-cultivation technology is presented that converts greenhouse gasses, CH_4 and CO_2, into microbial biomass. The methanotrophic bacterium, *Methylomicrobium alcaliphilum* 20z, was coupled to a cyanobacterium, *Synechococcus* PCC 7002 via oxygenic photosynthesis. The system exhibited robust growth on diverse gas mixtures ranging from biogas to those representative of a natural gas feedstock. A continuous processes was developed on a synthetic natural gas feed that achieved steady-state by imposing coupled light and O_2 limitations on the cyanobacterium and methanotroph, respectively. Continuous co-cultivation resulted in an O_2 depleted reactor and does not require CH_4/O_2 mixtures to be fed into the system, thereby enhancing process safety considerations over traditional methanotroph mono-culture platforms. This co-culture technology is scalable with respect to its ability to utilize different gas streams and its biological components constructed from model bacteria that can be metabolically customized to produce a range of biofuels and bioproducts.
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1. Background

Microbial biomass is a clean, renewable energy source that can significantly diversify and sustain future energy and transportation fuel requirements (Elliott et al., 2015; Tian et al., 2014). Production of microbial biomass and targeted bioproducts often depends upon costly substrates, such as glucose or other sugars, that limit the economic viability of the process (Kumar et al., 2012; Rodriguez et al., 2014). In contrast, various trends in algal cultivation have promised to develop renewable bioprocesses that convert inorganic carbon (CO_2/HCO_3^-) into microbial biomass using solar energy; however, process viability is often constrained by low biomass productivities and the inherent limitations of photosynthetic efficiencies (Grobbelaar, 2010; Quinn and Davis, 2015). Natural gas or biogas derived CH_4 represents an alternative, energy rich carbon source for generating microbial biomass and bioproducts (Kalyuzhnaya et al., 2015; Sheets et al., 2016) yet O_2 demands and mass transfer limitations remain challenging aspects for developing viable and safe bioprocesses (Rishell et al., 2004). Co-cultivation platforms of photoautotrophic and methanotrophic microbes represent a unique and promising option for concurrent capture of CO_2 and CH_4 within an integrated system (van der Ha et al., 2012)

CH_4 and CO_2 have the largest contributions to atmospheric radiative forcing caused by anthropogenic greenhouse gasses (GHG) (Robertson et al., 2000). Natural gas is an abundant resource that has played a central role in global energy production. Much of the natural gas that is co-produced with oil recovery (5 quadrillion BTU, ~5% of annual production) is unused, flared or vented representing a major GHG contribution that could be redirected and consumed as microbial feedstock (Fei et al., 2014). Biogas represents another major source of CH_4 and is an important renewable

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energy source that can be upgraded to a gaseous transportation fuel or combusted to generate electricity. However, biogas utilization is constrained because of its high upgrade costs and presence of contaminants such as H_2S and organosilicon (i.e., siloxanes). A large fraction of biogas produced is also being flared or vented into the atmosphere, representing yet another wasted product that could be redirected into microbial biomass and targeted bioproducts.

Many state-of-the-art biotechnologies are seeking to capitalize on the productivity and stability advantages gained though co-cultivation of microbial consortia (Bernstein and Carlson, 2012; Bernstein et al., 2012; Gilmore and O'Malley, 2016; Lindemann et al., 2016). This study presents new bacterial co-culture platform for concurrent conversion of CH_4 and CO_2 into biomass by employing a cyanobacteria-methanotroph binary culture. The co-culture technology is based upon robust metabolic coupling between oxygenic photosynthesis and methane oxidation. Continuous steady-state operation can be achieved based on light and O_2 limitation of the complimentary trophic partners, resulting in balanced and, ultimately, self-regulated growth. This co-culture has been demonstrated using various mixtures of CH_4, CO_2, O_2 as well as raw biogas to test performance on different GHG feedstocks. Importantly, the bacteria used to build this co-culture are amenable to metabolic engineering which renders the platform flexible and scalable for the production of different bioproducts on cost-effective, renewable CH_4 feedstocks.

2. Materials and methods

2.1. Bacterial strains and media

Synechococcus sp. PCC 7002 and *Methylomicrobium alcaliphilum* 20z were grown under co-culture and axenic conditions. All cultures were grown in one of two previously described minimal salts media, P-medium (Khmelenina et al., 1999) or A-plus medium (Stevens and Porter, 1980).

2.2. CH_4 feedstocks

Raw biogas was collected from an anaerobic digester (located in Outlook WA, USA) that was operated on dairy farm waste. The biogas composition was 58% CH_4, 42% CO_2, 0% O_2, and 0.3% H_2S as measured by a Landtec biogas 5000 m (Landtech, Dexter, MI). A synthetic natural gas feed stream of 80% CH_4, 17% N_2, and 3% CO_2 was made by mixing pure gas flows through calibrated rotameters.

2.3. Batch cultivation

The maximum specific growth rates on various biogas, CH_4, CO_2 and O_2 mixtures were obtained using sealed 30 ml Balch tubes incubated under constant 250 μmol photons m^{-2} sec^{-1} (fluorescent light). Each tube was charged with 8 ml A-plus medium (pH 8.0), sparged with an appropriate gas mixture and sealed with the respective gas in the head-space maintained at 1 ATM. The optical density (OD_{730nm}) was measured over a 96 h period using a Spectronic 20D+ spectrophotometer (Thermo Spectronic, Thermo Fisher Scientific, Waltham, MA). Each culture Balch tube was inoculated to a starting $OD_{730nm} = 0.028 \pm 0.005$. Co-cultures were inoculated with a 1:1 ratio of *M. alcaliphilum* and *Synechococcus* 7002, respectively.

2.4. Photobioreactor cultivation

Continuous cultivation was performed under chemostat mode by previously described methods (Beliaev et al., 2014; Bernstein et al., 2015). Briefly, di-chromatic (680 and 630 nm LEDs) photobioreactors were operated as light and O_2 limited chemostats using the New Brunswick BioFlo 3000 fermenter charged with a 5.5 L working volume diluted at a 0.03 h^{-1}, 30 °C, pH 8.0 and controlled for constant incident and transmitted irradiance (250 and 10 μmol photons m^{-2} s^{-1}, respectively). Cells were never exposed to dark conditions during these experiments. The control volume was sparged at 0.25 L min^{-1} with a synthetic natural gas mixture, 80% CH_4, 17% N_2, 3% CO_2. Steady-state biomass concentrations were measured directly as ash-free cell dry weight (g_{CDW} L^{-1}) as previously reported (Pinchuk et al., 2010). The volumetric gas mass transfer coefficient, $k_La = 5.32$ h^{-1}, was directly measured in the reactor under abiotic conditions by the unsteady re-aeration technique. Dissolved O_2 concentration in the reactor was measured with a Clark O_2 electrode (InPro® 6800Series, Mettler Toledo International Inc., Columbus, OH). The *in situ* net rate of O_2 production was calculated from the steady-state mass balance through the bioreactor control volume (Eq. (1)).

$$q_{O_2}x = D\left([O_2^{in}] - [O_2]\right) + k_l a\left(k_H p O_2^{in} - [O_2]\right) \tag{1}$$

The specific rate of O_2 production q_{O_2} multiplied by the biomass concentration (x) is interpreted here as the net rate of O_2 production during photosynthesis (Bernstein et al., 2014) and is a function of the dilution rate (D), k_La, dissolved O_2 concentration ($[O_2]$) and Henry's law partitioning coefficient ($k_H = 1.08$ mM atm^{-1}). The specific rate of biomass production (q_x; Cmmol biomass h^{-1} g_{AFDW}^{-1}) was calculated by assuming the molecular weight of ash free dry biomass (AFDW) to be 24.59 g_{AFDW} $Cmol^{-1}$ (Roels, 1980). The net rate of photosynthesis was also determined as a function of incident irradiance (P-I curve) by collecting axenic *Synechococcus* 7002 cells from the bioreactor and measuring the volumetric O_2 production rates as a function of 'white light' (tungsten incandescent) inside an oxygraph chamber (Hansatech, Norfolk, UK) coupled to a 2π quantum sensor (LI-210SA Photometric Sensor, LI-COR Biosciences, Lincoln, NE). The P-I curve was fit to the Jassby-Platt (Eq. (2)) using a parametric nonlinear regression (Jassby and Platt, 1976).

$$P = P_{max} \cdot \tanh\left(\frac{I_i}{I_k}\right) \tag{2}$$

The net volumetric rate of oxygenic photosynthesis (P) was estimated as a function of incident irradiance (I_i) by fitting to the single response variable (I_k), which represents the theoretical saturating irradiance of photosynthesis. The maximum net volumetric rate of oxygenic photosynthesis (P_{max}) is a constant that was directly measured from the P-I curve.

2.5. Flow cytometry and imaging

The relative abundances of *Synechococcus* 7002 and *M. alcaliphilum* cells were determined by flow cytometry using a BD Influx Fluorescence Activated Cell Sorter (FACS, BD Biosciences, San Jose, CA). Upon harvesting, the cells were immediately treated with 50 mM Na_2EDTA (Sigma-Aldrich) and gently pipetted to disrupt large aggregates and then fixed with 2% paraformaldehyde. Using the 488-nm excitation from a Sapphire LP laser (Coherent Inc., Santa Clara, CA) at 100 mW, samples were analyzed using a 70-μm nozzle. Optimization and calibration of the FACS was performed before each analysis using 3 μm Ultra Rainbow Fluorescent Particles (Spherotech, Lake Forest, IL). The ratio of the two distinct populations of cells within a mixed microbial community were identified from 50,000 recorded cells via size and complexity gates using FloJo (FloJo, LLC Ashland, OR) flow cytometry software. Microscopic images were acquired on a Zeiss LSM 710 Scanning Confocal Laser Microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany) equipped with an alpha Plan-Apochromat 100x/1.46 Oil DIC M27 objective. *M. alcaliphilum* cells were visualized by SYBR Gold Nucleic Acid Gel Stain (Invitrogen, Grand Island, NY) at 529–555 nm. *Synechococcus* 7002 was visualized by phycocyanin/chlorophyll auto-fluorescence measured at 655–685 nm. Structured illumination microscopy (SIM) was performed on a Zeiss Elyra S1 microscope using an alpha Plan-Apochromat 100x/1.46 oil-immersion objective. Excitation of SYBR Gold (Thermo Fisher) and phycocyanin/chlorophyll autofluorescence was achieved using 488 nm and 642 nm laser excitation, respectively. Emission band-pass filters were 495–550 nm and Long Pass 655 nm, respectively. The two excitation/emission color channels were recorded consecutively. Within each color channel, the raw data contained 3 rotations, 5 phases and 0.1 μm spacing z stack images. The super-resolution images were then reconstructed from raw images using ZEISS Efficient Navigation (ZEN) 2012 software to provide 2D and 3D projections or Volocity (Perkin Elmer). Confocal and SIM images were used to obtain the cell size measurements made along the major and minor axis. Cell volume calculations for each organism were carried out using the equation for an ellipsoid, $V = \frac{4}{3}\pi a^2 b$, where a is the minor axis, and b is the major axis. The relative biomass content of each organism in the co-culture was calculated using the Eq. (3), where v_1 and v_2 are the cell volumes of each organism, r_1 and r_2 are the measured population ratios.

$$B_1 = \frac{v_1 r_1}{(v_1 r_1 + v_2 r_2)} \tag{3}$$

3. Results and discussion

3.1. Oxygenic photosynthesis drives conversion of biogas to biomass

M. alcaliphilum and *Synechococcus* 7002 co-cultures, that were fed raw biogas, sustained longer periods of growth and achieved higher biomass concentrations compared to the *M. alcaliphilum* axenic control (Fig. 1). The axenic *Synechococcus* 7002 cultures were viable and grew rapidly on raw biogas as the sole carbon source. *Synechococcus* 7002 reached stationary phase rapidly

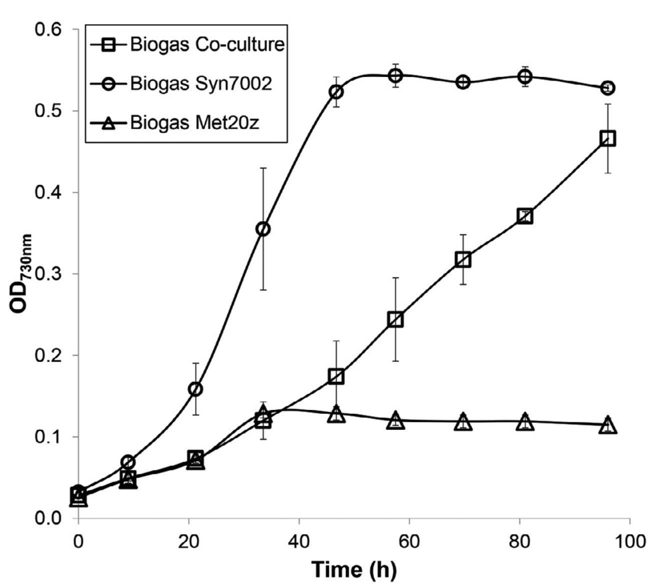

Fig. 1. Dynamics of batch growth supported on raw biogas used as the sole carbon source. Co-culture performance on biogas compared to axenic controls of *M. alcaliphilum* (Met20z) and *Synechococcus* 7002 (Syn7002), respectively. Each data point represents the mean from triplicate experimental measurements of optical density; error bars represent ±1 standard deviation.

(~45 h) likely due to depletion of CO_2. The co-culture continued to grow through the duration of the 96 h experiment and showed linear growth with time that was a signature of O_2 limitation. Longer periods of co-culture growth were sustained by the intrinsic supply of photosynthetically derived O_2 required for CH_4 oxidation.

The composition of gas effected the growth properties of each axenic and co-culture batch experiment (Fig. 2). The highest specific growth rate and maximum OD_{730} was observed for axenic *Synechococcus* 7002 cultured in biogas, 0.061 ± 0.002 h^{-1} and 0.545 ± 0.006, respectively. Supplementation of 20% O_2 to the biogas decreased the specific growth rate of axenic *Synechococcus* 7002 by 49%. Incubations under a 97% CH_4 and 3% CO_2 headspace resulted the highest co-culture specific growth rates and maximum OD_{730}, 0.050 ± 0.005 h^{-1} and 0.532 ± 0.017, respectively. The co-culture exhibited higher maximum biomass loads (OD_{730}) but slightly lower specific growth rates as compared to the axenic *M. alcaliphilum* controls cultured under analogous conditions. Increased CO_2 supplementation (to 50% in pure CH_4) decreased the growth performance of all axenic and co-cultures as compared to biogas or higher CH_4 composition feeds indicating that high inorganic carbon concentrations inhibits microbial growth in the system.

These batch results, obtained on biogas and variable gas compositions, highlight the versatility of the cyanobacteria-methanotroph co-culture technology while utilizing a renewable CH_4 and CO_2 feedstock for producing microbial biomass. This technology harnesses a metabolic coupling interaction between oxygenic photoautotrophs and methanotrophs that is naturally occurring in many terrestrial and aquatic ecosystems (Le Mer and Roger, 2001; Milucka et al., 2015). The bioengineering applications of coupled microbial photosynthesis and methane oxidation are promising and have previously been investigated using waste-water enrichment cultures (Van der Ha et al., 2011) and co-cultures driven by non-axenic green algae (van der Ha et al., 2012). The current study differs from previous investigations in multiple respects. First, this new co-culture technology is constructed from pure cultures of genetically tractable bacteria, *Synechococcus* 7002 and *M. alcaliphilum* 20z (Ojala et al., 2011; Xu et al., 2011a). Also, previous co-culture studies utilized a synthetic biogas substrate (van der Ha et al., 2012) as compared to the tests presented here on raw biogas feedstock, sourced from an anaerobic digester operated on dairy waste. The current system displayed robust growth on raw biogas, containing trace H_2S, that can inhibit microbial growth (Ge et al., 2014).

3.2. Continuous cultivation on a synthetic natural gas mixture

The co-culture was supported under steady-state conditions in a LED-illuminated chemostat sparged with the synthetic natural gas mixture, 80% CH_4, 17% N_2 and 3% CO_2. The total biomass load, held at a dilution rate of 0.3 h^{-1}, was 0.68 ± 0.01 g_{cdw} L^{-1} (mean of 5 measurements over 3 residence times). The species-specific abundances and steady-state specific rates were determined via cell counting (FACS) and microscopy-enabled measurements of cell sizes (Fig. 3). The steady-state mass fractions of *Synechococcus* 7002 and *M. alcaliphilum* were 0.562 ± 0.003 and 0.438 ± 0.01, respectively.

During steady-state co-cultivation, the source of O_2 required for CH_4 oxidation and *M. alcaliphilum* growth, was supplied by oxygenic photosynthesis. The dissolved O_2 concentration, inside the chemostat control volume, was negligible (0.56 ± 0.01 μM). Hence, *M. alcaliphilum* was O_2-limited and thereby limited by the rate of photosynthesis performed by light-limited *Synechococcus* 7002 cells. Under this metabolically coupled steady-state, the rate of O_2 consumption was equal to the net rate of photosynthetic

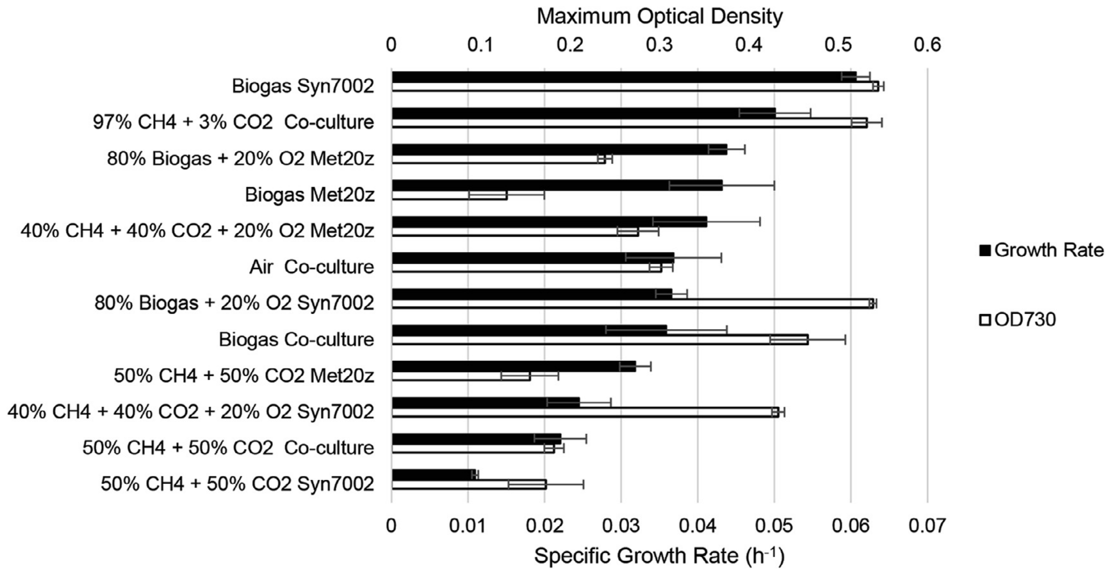

Fig. 2. Batch growth performance on variable gas mixtures use as the sole carbon source for the co-culture and axenic controls of *M. alcaliphilum* (Met20z) and *Synechococcus* 7002 (Syn7002), respectively. Each data point represents the mean of triplicate experimental measurements for either the specific growth rate or maximum optical density; error bars represent ±1 standard deviation.

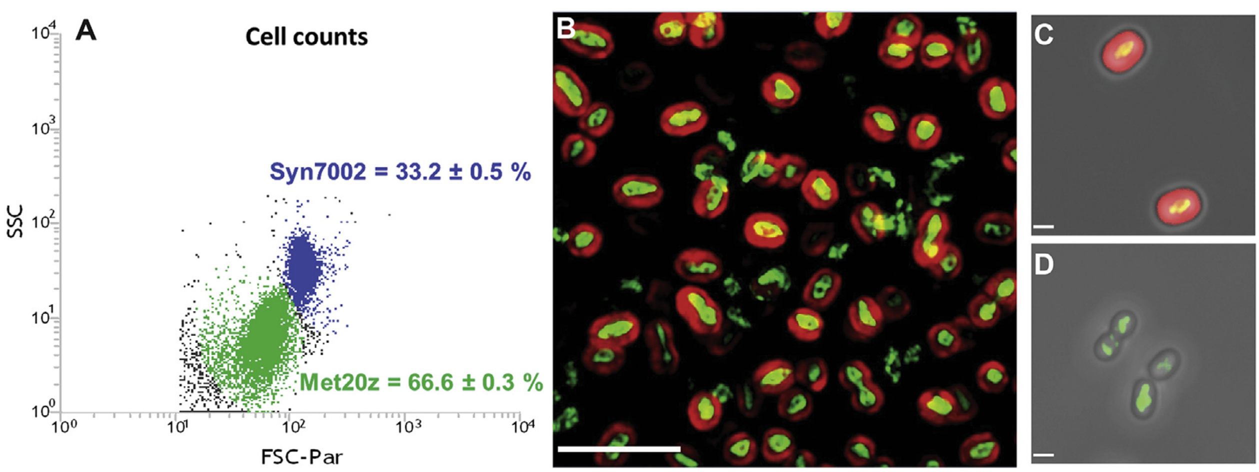

Fig. 3. Fluorescence-activated cell sorting (FACS), structured illumination microscopy (SIM) and confocal microscopy data/images used to determine cell counts and sizes. A) Results from FACS-enabled enumeration of each cell type; side scatter (SSC) plotted against forward scatter (FSC). Green and blue data points represent *M. alcaliphilum* (Met20z) and *Synechococcus* 7002 particles (Syn7002), respectively. B) SIM image of the co-culture; green represents nucleic acid stain (SYBR Gold) and red is chlorophyll autofluorescence specific to cyanobacterium *Synechococcus* 7002; the scale bar represents 5 μm. C) A representative confocal image frame of *Synechococcus* 7002 (1 of 100) used to calculate the major and minor axis dimensions of the cell. D) A representative confocal image frame of *M. alcaliphilum* (1 of 95) used to calculate the major and minor axis dimensions of the cell. The scale bars in images C and D represent 1 μm.

production. The specific rate of O_2 production (0.781 ± 0.002 mmol O_2 g_{cdw}^{-1} h^{-1}) was calculated from the dynamic recovery of dissolved O_2 concentration in the absence of CH_4 oxidation, by replacing CH_4 flow with a volumetric equivalent of N_2 gas (Fig. 4A).

The specific rate of co-culture biomass production, on a carbon mole (Cmol) basis, was 1.22 Cmmol g_{cdw}^{-1} h^{-1}. The minimum specific rates of CO_2/HCO_3^- and CH_4 uptake were calculated to be 0.686 ± 0.003 and 0.535 ± 0.001 Cmmol $g_{cdw\_total}^{-1}$ h^{-1} for *Synechococcus* 7002 and *M. alcaliphilum*, respectively (Fig. 4B). Based on the metabolic coupling requirement, it is reasonable to assume that the *M. alcaliphilum* biomass production rate is controlled by the input of actinic light at environmental conditions near the experimental set points and thus its growth can be modelled as a simple function of incident irradiance (I_i). Eq. (4) describes this relationship and makes use of parameter estimates obtained by the data presented in Fig. 5.

$$R_{Met20z} = Y_{X/O2} \cdot P_{max} \cdot \tanh\left(\frac{I_i}{I_k}\right) \quad (4)$$

The volumetric biomass productivity of *M. alcaliphilum* (R_{Met20z}) is dependent on the biomass-to-O_2 yield, ($Y_{x/O2}$) that was assumed to be constant at the measured value of 0.68 Cmol biomass produced per mole O_2 consumed (Fig. 5C). This yield constant was predicted from the mid-log batch phase of axenic *M. alcaliphilum* growth in the photobioreactor supplemented with O_2 at a partial pressure of 0.17 ATM and it showed 99.4% agreement with the independent measurement taken from the co-culture steady-state, 0.683 ± 0.002 Cmol *M. alcaliphilum* biomass produced per mole O_2 consumed. The maximum volumetric rate of oxygenic photosynthesis (P_{max}) and theoretical saturating irradiance (I_k) were parametrized from the *Synechococcus* 7002 P-I curve (Fig. 5A and Eq. (2)) and were 12.55 μM O_2 min^{-1} and 337 μmol photons m^{-2} s^{-1}, respectively. The model was able to

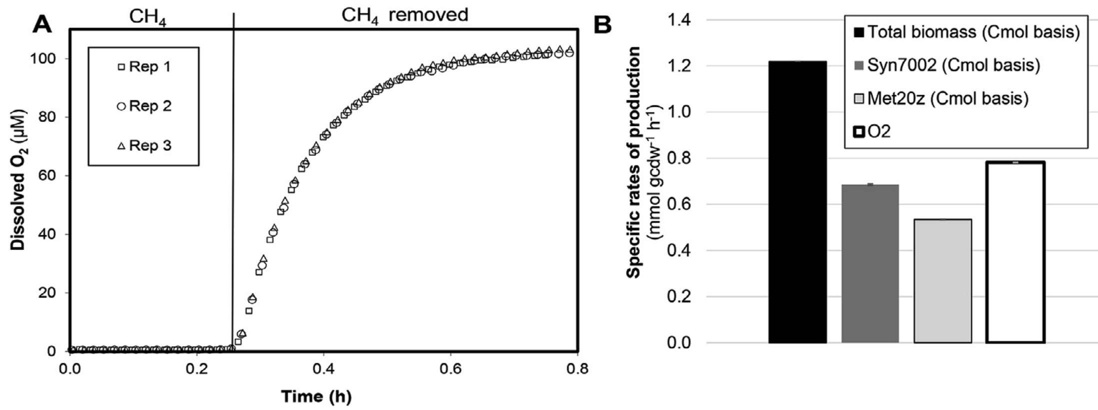

Fig. 4. Steady-state chemostat co-cultures supported on the synthetic natural gas mixture. A) At steady-state, the *M. alcaliphilum* population was O_2 limited indicating that their rate of consumption was equal to the rate of photosynthetic production. This rate was calculated from the dissolved O_2 concentration, in the absence of CH_4 oxidation, by interrupting CH_4 flow and replacing it with a volumetric equivalent of N_2 gas. The CH_4 removal perturbation was repeated three times from standing steady-state. B) Steady-state specific rates of production of biomass (Cmol basis) and net photosynthetic O_2 production (equivalent to *M. alcaliphilum* O_2 consumption rate). Each value was calculated with mean values obtained from a minimum of 4 replicate measurements taken at steady-state and error bars represent ±1 standard deviation.

make a relatively accurate prediction for the *M. alcaliphilum* biomass productivity by accounting for the incident irradiance (260 µmol photons $m^{-2}\,s^{-1}$) and control volume (5.5 L) used to establish the metabolically coupled steady-state in the chemostat photobioreactor. The predicted volumetric biomass productivity for the *M. alcaliphilum* component of the population was 1.82 Cmmol h^{-1} which showed 90% agreement with the independently obtained bioreactor measurement of 2.026 ± 0.006 Cmmol h^{-1}. Similarly, the net volumetric rate of photosynthesis predicted by the Jassby-Platt term (Eq. (2)) was 2.68 mmol $O_2\,h^{-1}$ which showed a 90.6% agreement to the independent measurement taken from the photobioreactor at steady-state, 2.962 ± 0.009 3.15 mmol $O_2\,h^{-1}$. The minor discrepancies between the predicted and measured productivities ($\leqslant$10% variance) are likely related to the difference in light quality used between the photobioreactor (dichromatic LED light) and the oxygraph chamber ('white' incandescent light). The upper bound for *M. alcaliphilum* biomass productivity corresponds to the theoretical saturating irradiance (I_k = 337 µmol photons $m^{-2}\,s^{-1}$) under the assumption that the observed metabolic coupling will continue to be controlled by O_2-limitation of methanotrophic growth at higher irradiances than the 260 µmol photons $m^{-2}\,s^{-1}$ that was explicitly tested in the chemostat. The predicted maximum *M. alcaliphilum* biomass productivity was 2.14 Cmmol h^{-1} and based on the saturating, hyperbolic shape of the P-I curve, obtained from *Synechococcus* 7002, this value is expected to remain constant with increasing irradiances beyond I_k. Similarly, the peak respiration rate for *M. alcaliphilum* was assumed to be equal to the maximum net rate of oxygenic photosynthesis of *Synechococcus* 7002 illuminated I_k and was estimated to be 3.15 mmol $O_2\,h^{-1}$.

Based on the experimental measurements, the illuminated areal biomass productivity of the system was 33.04 ± 0.55 Cmmol $h^{-1}\,m^{-2}$ and the individual productivities of *Synechococcus* 7002 and *M. alcaliphilim* were, 18.57 ± 0.09 and 14.47 ± 0.04 Cmmol $h^{-1}\,m^{-2}$, respectively. Photosynthetic cultures are often expressed on an areal basis to reflect their intended application in solar or hybrid LED/solar lighting. Although the bench-scale chemostat demonstration of this co-culture technology was not optimized for solar-irradiance inputs, the LED-illuminated prototype reactors achieved a relatively high biomass productivity 19.5 ± 0.3 $g_{cdw}\,m^{-2}\,day^{-1}$. This high areal productivity was achieved despite the conservative dilution rates chosen for proof-of-concept demonstration. Further process optimization has great potential to achieve the 25 $g_{cdw}\,m^{-2}\,day^{-1}$ target set forth by a recent report outlining the biomass production goals of the U.S. Department of Energy (Davis et al., 2016). Future optimization and scaling of this, and similar processes, will need to consider other factors such as dynamic environmental conditions (specifically diel cycles for solar powered systems) and the biological responses to minor components in raw natural gas. Although the components of natural gas other than CH_4 differ by reservoir, they are typically composed of a mixture of volatile organic (VOC) compounds including paraffins, naphthenes and aromatics that have the potential to inhibit bacterial growth and metabolism (Abuhamed et al., 2004; Gilman et al., 2013). VOCs thereby represent yet another reduced carbon source that might be utilized as a substrate in future expansions of this co-culture toward more complex, multi-functional microbial consortia.

3.3. Future directions

This co-culture technology, as currently reported, is a prototype platform that can be customized to produce a range of bioproducts concurrently with microbial biomass. Cyanobacterium *Synechococcus* 7002 is of keen interest to industrial biotechnologists as a metabolically tractable (Xu et al., 2011b), highly productive microbe (Bernstein et al., 2016) that has been demonstrated as a chassis for producing terpenoids (Davies et al., 2014), sugars (Xu et al., 2013) and fatty-acid biodiesel precursors (Kuo and Khosla, 2014). Similarly, *M. alcaliphilum* and related methanotrophs are amenable to metabolic engineering and synthesis of value added bioproducts such as single cell proteins, poly-3-hydroxybutyrate (PHB), methanol and fatty-acid biodiesel precursors (Kalyuzhnaya et al., 2015). Microbial biomass itself is a promising bioproduct that can be used as feedstock in modern catalytic conversion technologies such as hydrothermal liquefaction (Chen et al., 2014; Elliott et al., 2015) and other thermochemical conversion methods (Balat et al., 2009) that produce biocrudes with comparable characteristics to petroleum crude oils (Vardon et al., 2011). In this manner, the ability of multi-species microbial consortia to collaborate for the efficient capture and conversion of CH_4 and CO_2 represents a tremendous opportunity for biotechnologists and engineers to continue investigating.

4. Conclusion

The results presented here highlight the versatility of a cyanobacteria-methanotroph co-culture technology capable of

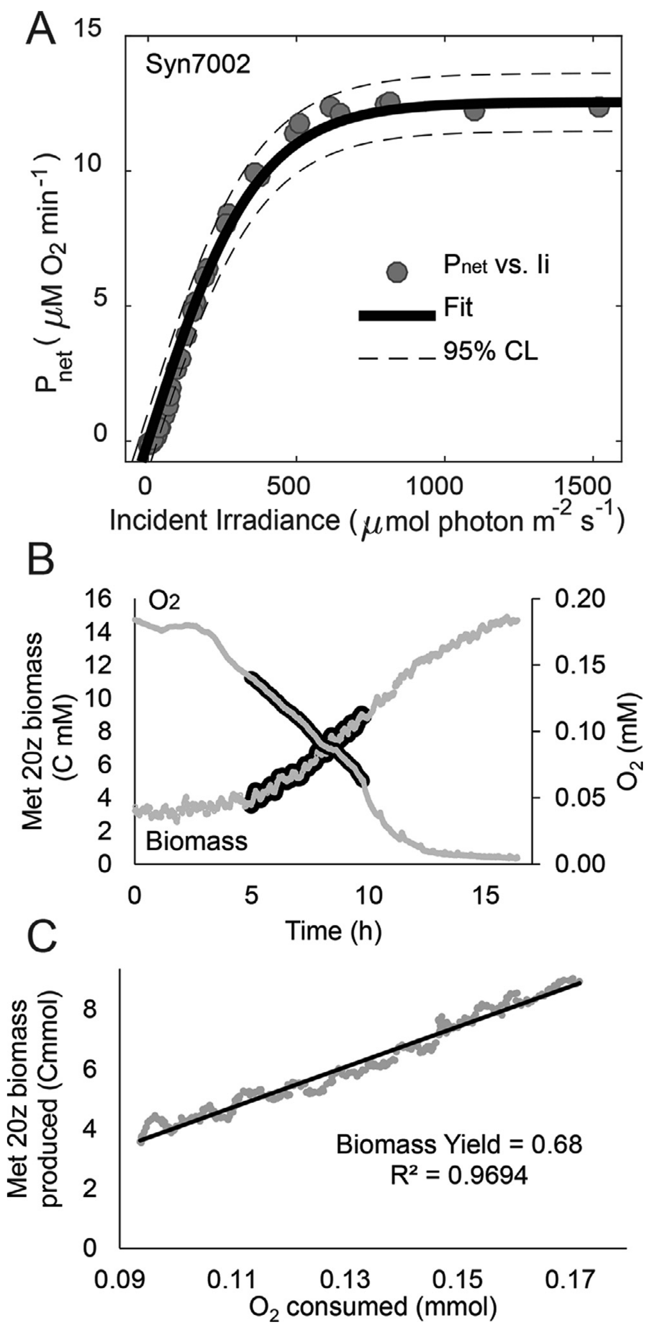

Fig. 5. Batch growth data for axenic *Synechococcus* 7002 (Syn7002) and *M. alcaliphilum* (Met20z) cultures used to parameterize coupled growth and photosynthesis kinetics. A) A P-I curve obtained on Syn7002 cells harvested from the bioreactor. All data points from biological duplicate measurements are plotted and fit with the Jassby-Platt model; dotted lines represent the 95% confidence limit bounds. B) Batch growth dynamics of Met20z showing the biomass concentration (on a carbon mole basis) and dissolved O_2; data points with dark black boundaries represent those in mid-log phase. C) The mid-log phase biomass concentration plotted against O_2-consumed; the linear slope of this relationship was used to calculate the biomass-to-O_2 yield for Met20z.

concurrently converting GHGs, CH_4 and CO_2, into microbial biomass. This technology is based upon robust metabolic coupling between oxygenic photosynthesis and CH_4 oxidation and demonstrates how pairing of a wild-type cyanobacterium and methanotroph can result in interspecies control of biological activity via exchange of process-limiting resources, specifically photosynthetically-derived O_2. This platform represents a new benchmark in co-culture technology that is scalable and flexible for engineering custom bioprocesses that concurrently remediate GHGs that are environmentally-toxic waste products generated by current energy production activities.

Conflict of interests

The authors have no conflicts of interest to declare.

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