Journal Pre-proof

Photo-driven heterogeneous microbial consortium reducing CO₂ to hydrocarbons fuel

Wenjie Xia, Rui Chen, Yang Li, Peike Gao, Chihong Li, Tianzhi Jin, John Ma, Ting Ma

PII: S0959-6526(21)03581-2

DOI: <https://doi.org/10.1016/j.jclepro.2021.129397>

Reference: JCLP 129397

To appear in: *Journal of Cleaner Production*

Received Date: 24 April 2021

Revised Date: 10 September 2021

Accepted Date: 15 October 2021

Please cite this article as: Xia W, Chen R, Li Y, Gao P, Li C, Jin T, Ma J, Ma T, Photo-driven heterogeneous microbial consortium reducing CO₂ to hydrocarbons fuel, *Journal of Cleaner Production* (2021), doi: <https://doi.org/10.1016/j.jclepro.2021.129397>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Ltd.

Credit Author Statement

Wenjie Xia: Conceptualization, Investigation, Writing - original draft, Methodology, Supervision, Funding acquisition. **Rui Chen:** Writing - review & editing, Methodology. **Yang Li:** Writing - review & editing; **Peike Gao:** Writing - review and editing. **Chihong Li:** Writing - review and editing. **Tianzhi Jin:** Investigation, Validation. **John Ma:** Data curation, Writing - review and editing. **Ting Ma:** Project administration, Supervision, Funding acquisition.

Photo-driven Heterogeneous Microbial Consortium Reducing CO₂ to Hydrocarbons Fuel

Wenjie Xia,^{*,a,1} Rui Chen,^{b,1} Yang Li,^c Peike Gao,^d Chihong Li^e, Tianzhi Jin^a, John
Ma^f, Ting Ma^{a,*}

^a Key Laboratory of Molecular Microbiology and Technology, Ministry of Education,
College of Life Sciences, Nankai University, Tianjin 300071, P.R. China;

^b Biotechnology Research Institute, Tianjin Academy of Agricultural Sciences, Tianjin
300381, P.R. China;

^c Tianjin Cancer Institute, Tianjin Medical University Cancer Institute and Hospital,
Tianjin 300060, P.R. China;

^d School of Life Science, Qufu Normal University, Jining 273165, P.R. China;

^e CCC SDC Green Environmental Engineering Company, Shanghai 200120, P.R.
China;

^f Materials and Process Simulation Center, California Institute of Technology,
Pasadena, California 91125, United States.

* Corresponding author:

Wenjie Xia, E-mail: wenjie.xia@nankai.edu.cn;

Ting Ma, Phone: Email: tingma@nankai.edu.cn

¹ These authors contributed equally to this work

Graphic abstract

Abstract

Carbon dioxide as feedstock for hydrocarbon synthesis attracts numerous efforts via the biological or chemical approaches. With the purpose of constructing a robust and highly-efficient platform for CO₂ bioconversion to fuel, we investigated a photo-driven bioprocess of directing CO₂ reduction to C₁-C₆ alkanes/alkenes discovered from the enriched microbial consortia. GC-MS analysis revealed that the maximum yield of total gaseous hydrocarbons was 169.66 uM with the average CO₂ reduction rate of 100.87 uM/day decreasing from 11573.39 uM to 5905.73 uM during 50 days culturing. The pathway of CO₂ bioconversion was proposed according to the 16S rRNA and metagenomic sequencing that rTCA and Wood-Ljungdahl were mainly contributed to CO₂ fixation, and nitrogenase was responsible for the hydrocarbon syntheses. Following these results, a versatile consortium-based platform was developed via the neural network method with the selected *Pseudomonas* sp., *Serratia* sp., *Candidatus* sp., *Clostridium* sp., *Enterococcus* sp., *Salmonella* sp., *Rhodospirillum* sp., *Thalassospira* sp., *Thioclava* sp., *Stenotrophomonas* sp. and *Desulfovibrio* sp., which showed the improved CO₂ reduction rate of 107.54 uM/day and the higher selectivity of C₁-C₄ alkanes than the original consortia. This study demonstrated that this artificially constructed consortium could be a promising platform for converting CO₂ to the diverse gaseous alkanes and alkenes.

Keywords:

Bioconversion; CO₂; Hydrocarbons; Photosynthesis; Microorganism.

1. Introduction

The increasing demands in energy along with the expected long-term decline in petroleum reserves require alternative technologies for the production of fuels and important industrial chemical intermediates. The incessant exploitation and utilization of fossil-derived resources released significantly large quantities of CO₂ with its huge emission over 30 Gigatonne annually (Kintisch 2015). Targeting the emission mitigation, CO₂ as the promising starting material for producing fuels or valuable chemicals, has attracted more and more attentions (Blanchet et al., 2015). To achieve sustainability of energy, it is environmentally and economically benefit to build the carbon skeletons of hydrocarbon fuels derived from CO₂. However, key challenge we face is that utilization of CO₂ has certain disadvantages as a reactant due to its inert, chemical non-reactive, and low Gibbs free energy properties.

There have been significantly extensive efforts proceeding to make this strategy become achievable (Otto et al., 2015; Spurgeon and Kumar., 2018). For conversion to hydrocarbon, conventionally, multiple steps are required, where CO₂ is firstly reformed to synthesis gas (CO and H₂), followed by the Fischer-Tropsch process to produce hydrocarbons or catalytic conversion to produce methanol and subsequent methanol-to-olefin (MTO) or methanol-to-gasoline (MTG) processes (Kondratenko et al., 2013). Recent state-of-the-art developments in CO₂ technologies via the catalytic hydrogenation, the photocatalytic and electrocatalytic process for providing hydrogen to reduce CO₂ to methanol, hydrocarbons or oxygenates, have been reported with great quantity in lab-scale by the energy source from electrics or solar (Cho et al., 2017).

However, the integrity of various catalysis process and the synthesis of novel catalysts in these processes remain great challenging, and continuously extract huge efforts, and some expensively advanced materials (such as ion liquids, semiconductor) are also encompassed, in order to improve the selective accuracy and conversion efficiency in such chemical processes. In biology, CO₂ conversion via photosynthesis to fix carbon into microalgae (Rashid et al., 2014), cyanobacteria (Liu et al., 2019) and the engineered bacteria (Ni et al., 2018) has been extensively studied for enormous potential in the production of fuels and important industrial chemical intermediates. However, these biological processes are not directly converting CO₂ to the expected hydrocarbons. Although the metabolic engineering of microorganisms offers a promising route for the replacement, it is limited to use glucose or other organics as feedstock to produce hydrocarbons (Liao et al., 2016). Recently, attempts of metabolic engineering of photosynthetic organisms have been made to assimilate CO₂ to hydrocarbons, but the metabolites are the aromatic hydrocarbons (Ni et al., 2018). Nitrogenase from microorganisms is recently attracted more efforts due to its capacity of reducing CO₂ to short hydrocarbons, while methane is dominantly detected (Fixena et al., 2016; Lee et al., 2018). Few of reports on the direct bioconversion of CO₂ to hydrocarbons without metabolic engineering, particularly the metabolites of the gaseous alkanes and alkenes, have been reported.

Referring to the concept of the consolidated bioprocessing (CBP), an ideal system, integrating the primary (CO₂ fixation) and secondary (post-step biosynthesis) fermentation in a single processing step, has been treated as a promising biological

strategy for CO₂ bioconversion (Shahab et al., 2020). However, the engineering of these complex reaction cascades into a single microbial host can create a substantial metabolic burden that limits the overall efficiency and productivity, especially when CO₂ is initiated as solo carbon source (Tsoi et al., 2018). Consequently, low carbon and energy fluxes remain a major challenge for CBP approaches based on genetically engineered microorganisms, which are often characterized by metabolic imbalances (Gupta et al., 2017; Shahab et al., 2020). While this limitation can be broken through by a metabolic division of labor (Tsoi et al., 2018) and extra energy introduction (Kondratenko et al., 2013). This phenomenon is inherently observed in natural consortia, where complex reaction cascades and biochemically difficult tasks are catalyzed by multiple species according to the principle of metabolic compartmentalization or metabolic integration (Johns et al., 2016). Each member of the consortium occupies its ecological niche and is specialized in the relay of carbon and energy flux or exchanges different beneficial molecules. However, the high complexity of natural consortia makes the targeted engineering of the desired function complicated and limits their industrial application for biochemicals production (Shahab et al., 2020). Alternatively, less complexity and more defined synthetic consortia can be engineered (Stephens et al., 2019), which have been impressively applied for CBP of recalcitrant substrates to produce alcohols, organic acids or ketones (Hill et al., 2017; Shahab et al., 2020). A major challenge in the design of such consortium is the requirement of overlapping tolerance ranges of several abiotic process parameters, which limits the selection of possible consortium members (Ben Said et al., 2020). Thus, the majority of

co-culture studies reported to date had been carried out using only two species with similar abiotic requirements. Few researches of the artificial consortium with more than two species have been reported (Hill et al., 2017; Liu et al., 2017). Furthermore, the population control and stable reproducible performance are challenging to achieve, especially for microbial consortia which mutual beneficial interactions are missing (Coyte et al., 2015). To tackle these challenges, our attempts focus on the enrichment of the heterogeneous consortia from the natural water or soil samples with CO₂ as sole carbon source under a consistent condition such as the specific temperature, solar light, etc., then an enriched culturing with the highest value of CO₂ reduction rate and the yield of the valuable metabolites will be selected as the blueprint to construct the stable consortium for CO₂ conversion to the specific molecule.

Here we demonstrate an enriched consortium that can directly convert CO₂ to light hydrocarbons (C₁-C₆ alkanes and C₂-C₃ alkenes) with high selectivity. Following the CBP strategy, the metabolic pathway of this enriched consortium is investigated via the analyses of metabolite profiles and metagenomic sequencing to explore the key microorganisms involving in the CO₂ conversion. Then we reconstruct an artificially heterogeneous consortium with these key microorganisms via neural network method and validate its performance on CO₂ conversion to the gaseous hydrocarbons. This provides a potential platform for the industrial production of fuel and chemicals from CO₂.

2. Materials and methods

2.1 Consortium enrichment of CO₂ conversion

500 ml of water samples, fetched from south California coast at Malibu pier (34.03 °N, 118.78 °W) and La Jolla cove (32.85 °N, 117.27 °W), were filtrated for cell collection. The phytoplankton biomass was removed following the description before culturing (Inoue et al., 2007). The cell was suspended in sterilized water and transferred into 1000 ml of the pyrex reaction vessel filled with 500 ml mineral salts medium (including g/L: 0.3 K₂HPO₄, 0.3 NaH₂PO₄, 0.05 MgSO₄, 0.01 FeSO₄, 1.0 CaCO₃, 0.1 NaNO₃). The vessel was sealed and purged with nitrogen gas. CO₂ was syringed into the vessel as carbon source, the final pressure was 29.29 psi (at 25 °C). The whole vessel was exposed to the white LED light (6 mol photons m⁻² s⁻¹) for culturing at 25 °C. Samples collected at 4th, 17th, 31st, 38th day during the culturing were fetched and labeled as S1, S2, S3, S4 respectively for further analysis. Controls without cell or LED light were set up for determining kinetics of CO₂ consumption and hydrocarbon synthesis.

2.2. Hydrocarbons determination

Hydrocarbons were analyzed by gas chromatograph equipped an 8600-WB5B 30M×0.53mm I.D.1.0u DB-5 Type MXT-5 capillary column (Yu et al., 2019). 1 ml of the headspace gas was syringed with 10:1 split mode (1.9 ml/min He flow), the testing program: initial temperature 35 °C, hold 7 min; ramp 15 °C/min to 180 °C, hold 7 min at 180 °C; ramp 20 °C min⁻¹ to 245 °C; hold 9 min at 245 °C. Hydrocarbon was qualified by a commercial standard gas (Airgas, USA). Quantification was completed by relating the GC peak area of the sample to the peak area of the standard alkane and alkene gas mixtures (Praxair, Geismar, LA). All samples were performed in triplicates and the

results were expressed as mean value. The ^{13}C labeled CO_2 were applied to track the carbon flux, the method referred to the supplementary method (Zhou et al., 2019).

2.3. DNA samples preparation

Total DNA was isolated from 30 mL of each sample using the QIAamp DNA Mini Kit according to the manufacturer's protocol. Quality and purity of the DNA extracts were evaluated by agarose gelelectrophoresis, NanoDrop ND-1000 Spectrophotometer and Qubit fluorometer. All DNA samples were stored at -20°C until further processing.

2.4 16S rRNA sequencing

Taxonomic assignments based on 16S rRNA profiling was carried out by using primer Bac336F/Bac806R for bacteria and Arc334F/Arc806R for archaea (Takahashi et al., 2014). Amplification primers were derived from standard Illumina adapters, and PCR reaction was carried out according to the method described previously (Jiang et al., 2015). Qualified libraries were sequenced by the Illumina MiSeq paired-end sequencing platform. QIIME toolkit (V1.8.0) was applied for analyzing the abundance and structures of microbial communities (Caporaso et al., 2015). A similarity threshold of 97% was set for clustered operational taxonomical units (OTUs) according to the Greengenes reference (DeSantis et al., 2015).

2.5 Metagenomic sequencing

Metagenomic libraries were prepared using the NEBNext Ultra DNA Library Prep Kit for each sample. After assessed by Agilent 2100 Bioanalyzer and the KAPA Library Quantification Kits, the libraries were sequenced by Illumina HiSeq 2500 platform in a 2×150 bp paired-end mode.

2.6. DNA sequence assembly and annotation

Trimmomatic (V0.35) (Bolger et al.,2015), MEGAHIT (Li et al.,2015), Prokka and Salmon programs were utilized for read trimming, *de novo* assembly, gene annotation and abundance calculation, respectively. Binning analysis was performed by Maxbin and MetaBAT2 to recover individual genomes (Seemann et al., 2014; Patro et al., 2017).

The NT database and corresponding Taxonomy databases were downloaded from NCBI and EBI websites, and all sequences derived from archaea, bacteria, fungi, viroids and viruses were extracted and used as a self-built dataset. The normalized abundance of each taxonomic level was calculated by butt-joint of annotated genes and their best-hits in the micro-NT database. For functional analysis, target genes, modules and pathways were collected from KEGG database (Wu et al.,2014). BLASTP ($1e^{-5}$) was used for homologous gene alignment between annotated genes and designated target genes. Only the best-hit was extracted for abundance accumulation and comparative analysis among samples.

Metagenomic sequencing of all samples generated 80.66 Gb raw reads in total. Subsequent read cleaning and *de novo* assembly resulted in 327.3 Mb of sequences in 334,039 contigs, with an average size of 980 bp and an N50 of 1,669 bp. Approximately 98.85% of clean reads could be mapped back to the assembled contigs. The statistics of sequencing data was tabulated in Table S1.

2.7. Heterogeneous consortium construction

The isolation of the specific microorganisms from the enriched consortium was conducted under previous condition. Beside CO₂, the various supplementary carbon sources were initially applied, such as alcohol (C₁-C₆), ketone (C₃-C₆), and methane. Each 25 ml of sample from the enriched consortium was centrifuged to collect cell, and transferred it into 50 ml ampoule bottle filled with 10 ml of mineral salts medium, then purged with CO₂. 20 ppm of alcohol (C₁-C₆), ketone (C₃-C₆), and methane were syringed into each paralleled bottle respectively. During culturing, the gaseous metabolites from each sample was analyzed by GC, and any sample with hydrocarbons ($\geq C_2$) detected was selected to proceed the isolation via the agar plate spreading with the related culturing medium and the supplementary carbon source, and then cultivated in CO₂ incubator. The sample supplemented with methane was cultured in desiccator filled with CO₂ and methane. Meanwhile, the selected samples were diluted by 10, 100, 1000 times with the autoclaved mineral medium, and re-cultured at the same condition. All pure microorganisms were conserved and then identified by 16S rRNA sequencing. Neural network of DOE (Design of experiment via JMP) was applied to reconstruct the heterogeneous consortium.

2.8 Availability of data

All raw reads of 16S rRNA and metagenomic sequencing had been deposited at Sequence Read Archive under accession number PRJNA577349 and PRJNA512068. The facultatively and obligately anaerobic strains were deposited as GeneBank access No. MN538968 to MN538983.

3. Results and Discussion

3.1. CO₂ reduction to Hydrocarbon

Among the various cultures, one enriched consortium showed the performances that CO₂ was obviously utilized at the average consumption rate of 100.87 uM day⁻¹ decreasing from 11573.39 uM to 5905.73 uM during 50 days culturing when exposed to the consistent LED light. The gaseous metabolite was the short-chain hydrocarbons including C₁-C₆ alkanes and C₂-C₃ alkenes, the original profiles of GC shown in Fig. S1. As calculated, the total reduction rate of CO₂ was 48.9%; the maximum yield of total accumulated gaseous hydrocarbons was 169.67 uM observed at 38th day; and the production selectivity of C₂-C₅ hydrocarbons was more than 80% (Fig. 1a). In decades, few of literatures have elaborated such direct process of CO₂ bioconversion to these hydrocarbons (Kondratenko et al., 2013). The tentative processes have been effectively achieved the hydrocarbons synthesis via the genetic manipulation on the specific bacteria, but CO₂ was not applied as sole carbon source (Kallio et al., 2013), or other metabolites (like aromatic hydrocarbons, alcohols, acids, aldehydes, etc.) were produced even accompanying with CO₂ reduction (Liu et al., 2019). Recent reports on the functional enzymes like aldehyde decarbonylase (AD) (Scrutton et al., 2017) and nitrogenase showed their attractive functions on hydrocarbon synthesis with using aliphatic acids or carbon monoxide as substrate (Fixena et al., 2016; Lee et al., 2018), however the total yield, production rate of hydrocarbons, and consumption rate of CO₂ were lower when compared with our process. ¹³C-labeled CO₂ was applied to track the carbon flux and confirm the carbon origin. In the light of GC-MS data shown in Fig. S2, the feeding of ¹³CO₂ resulted in the incorporation of isotopic labelling into light

hydrocarbons as indicated by the molecular ion, which was increased by 1 to 6 for each alkane or alkane. The fragment ions were also increased by 1 to 5.

For each hydrocarbon, the methane production was primarily detected at early stage of our bioprocess, and reached the maximum value (66.22 μM) at 31st day (Fig.1b). During the period of 4th to 17th day, the production rate of methane was highest at 3.99 $\mu\text{M}/\text{d}$. Methane was generally produced by Methanogen under obligatorily anaerobic condition (Zhang et al., 2021), while was detected with the present of oxygen in our bioprocess (Fig.1a). It indicated the involvement of the other pathway in the methane production. Except methane, other gaseous hydrocarbons were negligibly detected before 31st day, but significantly observed after long-time cultivation coupling with the decreasing of methane concentration. n-Butane obtained the maximum value of 44.09 μM at 38th day, which was approximately 42.68-fold of ethane, 2.47-fold of propane, 4.58-fold of i-butane, 3.88-fold of i-pentane, 1.76-fold of n-pentane, 17.72-fold of i-hexane, and 53.64-fold of n-hexane (Fig. 1b). None of hydrocarbons was observed in the control samples. In addition, the isomer-type of alkane was less than the normal-alkane in our bioprocess. This trend showed the conformity with the theory of classic geochemistry that the ratio of the isomer-type against the normal-type generated in biological process was generally less than 1 in contrast with the geothermal process (Zhou et al., 2010). These results demonstrated the natural consortia have the significant ability to transform CO_2 and solar energy to the organic chemicals.

3.2. Strcuture and function of the enriched consortium

Among them, 12 OTUs were accounted for 98% to 99% of the total consortium, and prevalently distributed in all samples (>0.5% of the retrieved sequences, Fig.2): *Proteobacteria*, *Firmicutes*, *Basidiomycota*, *Actinobacteria*, *Ascomycota*, *Fusobacteria*, *Chytridiomycota*, *Tenericutes*, *Synergistetes*, *Mucoromycota*, *Cyanobacteria* and *Euryarchaeota*. Among them, *Proteobacteria* (>75% of the retrieved sequences) was accounted for dominate abundance in all samples. Its subdivision, including *alphaproteobacteria*, *betaproteobacteria*, *gamaproteobacteria*, *deltaproteobacteria* and *epsilonproteobacteria*, were detected in all samples. Particularly, *gamaproteobacteria* showed the obviously higher percentage which was increased from 39.88% in S1 to 96.35% in S4 during the culturing. *Gamaproteobacteria* has been extensively reported that the majority of this subdivision are capable of photoautotrophic pathway or chemolithoheterotrophic pathway for CO₂ fixation (Schirmer et al., 2010). *Cyanobacteria*, *Ignavibacteriae*, *Chlorobi*, and *Bacteroidetes* were only detected in sample S1, which have been well-elaborated the capacity of CO₂ fixation, nitrogen fixation, and hydrogen production via photoautotrophy or chemolithoautotrophy, however the phyla (like *Ignavibacteriae*, *Chlorobi*, etc) were sensitive to oxygen (Hiras et al., 2016). Oxygen was possibly generated from the oxygenic photoautotroph like *Cyanobacteria* (data shown in Fig. 1a). Consequently, the abundance of these oxygen-sensitive and CO₂-fixing phylotypes was lower than others, even cannot be detected during the culturing, which indicated *gamaproteobacteria* and *cyanobacteria* made dominant contributions to CO₂ fixation.

The gene catalogues were annotated by NR, KEGG and eggNOG based on the

metagenomic sequencing (Table S2). Protein sequences were analyzed to statistically identify protein categories of each sample. According to the functional categories, the predicted genes, assigned to the category of the energy production and conversion, had the highest proportion (Fig. 3). Regarding the unique KEGG orthologue group (KO) profile, the functional structures of four samples were obviously changed during culturing, and the diversity in sample S3 and S4 were lower than the sample S1 and S2 (Fig. s3). At pathway level, the subculture enriched Kos were significantly distributed in the category of the carbon fixation and hydrocarbon synthesis. Particularly, the abundance of the total genes encoding the carbon fixation enzyme in sample S1 and S2 was approximately 2 folds in sample S3 and S4 (Fig. s3b). It also indicated that CO₂ reduction was indeed occurred to creat energy or produce precursors for hydrocarbon synthesis at early stage of the culturing.

3.3. Pathways and Microorganisms of CO₂ fixation

Six natural CO₂-fixation pathways and the relevant key genes, including the Calvin-Benson-Bassham (CBB) cycle, the 3-hydroxypropionate cycle, the Wood-Ljungdahl (WL) pathway, the reductive tricarboxylic acid (rTCA) cycle, the dicarboxylate/4-hydroxybutyrate cycle, and the 3-hydroxypropionate-4-hydroxybutyrate cycle, have been analyzed to predict the potential pathway for such CO₂ conversion process based on KEGG database (Jones 2008). As acknowledged, the CBB cycle, the 3-hydroxypropionate cycle, and 3-hydroxypropionate-4-hydroxybutyrate cycle were operated under aerobic condition, while the others were anaerobic pathways (Jones 2008). Given all six pathways completely operated in our

bioprocess, the transition of the pathways must be occurred during culturing, as the evidence of the oxygen kinetics shown in Fig.1a. Based on the data of metagenomic sequencing, it presented that CBB cycle, rTCA cycle and WL pathway were completed, while the others were incomplete in all samples (Fig. S4). Key genes of these completed pathways, namely RubisCO for CBB cycle (Ni et al., 2018), ATP citrate lyase for rTCA cycle (Chen et al., 2019) and CO dehydrogenase/acetyl-CoA synthase for WL pathway (Doukov et al., 2002), were detected and showed the significant abundance in all samples (Fig. 4a). It demonstrated that the enriched consortium embodied the ability of autotrophic carbon fixation. During the culturing, the gene abundance of CBB cycle was decreased, while the gene abundance of rTCA cycle and WL pathway were increased (Fig. 4b). This phenomenon indicated that CO₂ reduction was initiated with CBB cycle, and switched to rTCA cycle and WL pathway, when oxygen was depleted or lower than the critical sensitive concentration (Fig. 1a).

At the phylum level, the structural dynamics of the enriched consortium suggested that *Proteobacteria* (51.5%-75.6%), *Firmucutes* (9.6%-19.7%), *Acinobacteria* (6.8%-14.8%) and *Euryarchaeota* (2.2%-7.8%) representing relative higher abundance were the main taxa responsible for CO₂ fixation (Fig. S5a-S5c). Numerous literatures have elaborated that CBB cycle was operated in *alpha*- and *gammaproteobacteria*, rTCA cycle had been found in representatives of the *epsilonproteobacteria*, and WL pathway was continually reported on *Proteobacteria*, *Firmucutes*, *Acinobacteria* and *Euryarchaeota* (Takai et al., 2005; Jones et al., 2019).

The contigs representing 76.8% of the total sequence data in all samples were

307 binned into the high-quality genome bins. 23 of the genome bins were identified as the
 308 prevalent OTUs. Phylogenetic tree was constructed and presented the placement of the
 309 selected bins in the relevant phylum or subdivision (Fig. S6). Among of them, *Nostoc*
 310 (bin 1), *Anabaena* (bin 2) and *Synechocystis* (bin 3) from the phylum of *Cyanobacteria*,
 311 belonging to the oxygenic phototrophs encoding RuBisCO, were detected and showed
 312 the abundance of less than 2% in all samples. But their abundance was relatively higher
 313 in sample S1 than that in other samples. *Nostoc* and *Anabaena* were only detected in
 314 sample S1, and *Synechocystis* was not detected in samples S3 and S4. Although the
 315 oxygenic phototrophs showed their presence, anoxygenic CO₂-fixation phylotypes
 316 were dominated, which included *Brucella* (bin 4), *Serratia* (bin 5), *Shigella* (bin 6),
 317 *Candidatus* (bin 7), *Chlorobium* (bin 8), *Azospirillum* (bin 9), *Azotobacter* (bin 10),
 318 *Vibrio* (bin 11), and *Simiduia* (bin 12) (Fig. S5c-S5e). It was the reason that oxygen
 319 concentration was not high and decreased after 4th day (Fig. 1a). Species from *Brucella*
 320 and *Vibrio* genus were the facultative CO₂-dependence bacteria that capable of
 321 producing amino acid, alcohol, aldehyde, etc (Shimamura et al., 1985; Pérez-Etayo et
 322 al., 2018), which can be used as precursors for hydrocarbon synthesis. Facultative
 323 *Candidatus* genus, capable of CO₂ fixation via CBB cycle or rTCA cycle, has recently
 324 been described an intra-aerobic function of generating the internal oxygen to facilitate
 325 bioreaction of those aerobic pathways (Rasigraf et al., 2014). This result provided the
 326 possibility of oxygenic bioreactions of hydrocarbon synthesis by aldehyde
 327 decarbonylase (AD) under anaerobic condition. Species from *Serratia* genus were well
 328 characterized by chemolithotrophic fixation of CO₂, which was verified by the presence

of RuBisCo, carbonic anhydrase, and carboxylases (Kumar et al., 2016).

As shown in Fig.4, the key gene abundance of rTCA cycle and WL pathway were increased during the culturing and higher than that in CBB cycle. *Chlorobium sp.* (bin 8), as anaerobic phototrophs fixing CO₂ via a rTCA cycle (Chen et al., 2019), was detected with increasing abundance in the enrichment starting from 17th day. However it was observed with the negligible percentage at the early stage of culturing. The archaeon *Halobacterium* (bin 13) was detected, which can capture light energy to assimilate CO₂ (Javor et al., 1988). Based on KEGG database, the specific species of *Halobacterium* expressed enzymes that can operate the WL pathway to fix CO₂.

WL pathway is regarded as the only pathway that fixes CO₂ while conserving energy as ATP in numerous, phylogenetically disparate and anaerobic *Bacteria* and *Archaea* (Berg et al., 2010). In the phylum of *Firmicutes* (shown in Fig.2), *Acetobacterium woodii*, *Moorella thermoacetica*, *Hydrogenotrophic methanogens*, *Clostridium aceticum*, and autotrophic sulfate-reducing prokaryotes inherently operated WL pathway (Jones 2008; Berg et al., 2010). As intermediate of WL pathway, CO was detected during the culturing, which was reduced from CO₂ by carbon monoxide dehydrogenase (CODH) (Doukov et al., 2002). Metagenomic analysis of genes encoding the enzymes CODH showed that the abundance of *Clostridium* (bin 8), *Carboxydotherrmus* (bin 14), *Rhodospirillum* (bin 15), *Pseudomonas* (bin 16) and *Methanosarcina* (bin 17) were more than 5% and increased during culturing. These microorganisms can be capable of fixing CO₂ via WL pathway (Adam et al., 2019). *Pseudomonas sp.* (bin 16), belonging to the *Gammaproteobacteria*, was reported its

ability of fixing CO₂ via WL pathway (Igarashi et al., 2019), and showed the abundance was accounted for more than 5% in all samples, and particularly more than 15% in sample S3 and S4. WL pathway of *Methanosarcina* (bin 17) in the phylum of *Euryarchaeota* have also been well documented (Diender et al., 2015).

3.4. Pathways and microorganisms of hydrocarbon synthesis

Recent advances in biofuels have revealed an assortment of enzymes that can catalyze fatty acids, alcohol, or aldehyde to hydrocarbons (Scrutton 2017; Zhou et al., 2018), and particularly elaborated the significant role of aldehyde decarbonylase (AD) and nitrogenase on the production of alkane or alkene (Khara et al., 2013). Generally, the carboxylic acid reductase (CARs) can catalyze the carboxylic acid to the corresponding aldehydes, then can AD convert the aldehydes to hydrocarbons. The species from the phylum of *Cyanobacteria*, which can express AD, had relatively lower abundance when compared with others (Fig. 2). *Vibrio* genus, belonging to *Gammaproteobacteria*, was similarly reported about the synthesis of alkanes via the AD catalysis, however only even-carbon-number and long-chain (>C₁₀) alkanes were produced (Park 2015). The activity of AD is oxygen-dependently triggered. Although the oxygen was detected (Fig. 1a), the metagenomic analysis showed that the gene abundance of AD was lower than nitrogenases in all samples (Fig. 5a). Therefore, the AD played the marginal role on the synthesis of gaseous hydrocarbons, and indicated that there was the other pathway for hydrocarbon production.

Nitrogenases have been extensively reported its ability of converting small molecules (such as cyanide ions, CO, acetate, butanol, butyrate, cyclohexane

373 carboxylate, ethanol, fumarate, succinate, formaldehyde or acetaldehydes) with the
 374 reductant to alkanes and alkenes under ambient conditions (Fixen et al., 2016; Lee et
 375 al., 2018). It included three homologous nitrogenase enzymes that documented as the
 376 Mo-nitrogenase, V-nitrogenase and Fe-only nitrogenase. In our study, the nitrogen gas
 377 was consumed at rapid decreasing rate of 200.88 $\mu\text{M}/\text{d}$ from 15859.38 μM to 12444.43
 378 μM during the 17 days culturing (Fig. 1a), which indicated the energetic activity of
 379 nitrogenase. Metagenesis analysis showed that the key gene abundance of nitrogenase
 380 was significantly higher in all samples, and increased during the culturing (Fig. 5a).
 381 Particularly, Mo-nitrogenase and Fe-only nitrogenase were accounted for more than 50%
 382 in all samples (Fig. 5b), which have been well-reported the performance of catalyzing
 383 the reduction of CO and CO₂ to hydrocarbons (Fixen et al., 2016). According to
 384 metagenomic analysis, *Shigella* (bin 6), *Candidatus* (bin 7), *Clostridium* (bin 8),
 385 *Azospirillum* (bin 9), *Azotobacter* (bin 10), *Vibrio* (bin 11), *Rhodospirillum* (bin 15),
 386 *Pseudomonas* (bin 16), *Desulfovibrio* (bin 18), and *Deinococcus* (bin 19), were
 387 dominantly observed in all samples (Fig. S7). These microorganisms were capable of
 388 secreting nitrogenase under aerobic or anaerobic conditions, especially Mo and Fe-only
 389 types (Fixen et al., 2016). Furthermore, the abundance of the facultative and obligate
 390 anaerobic microbes, like *Clostridium*, *Pseudomonas* and *Desulfovibrio*, were
 391 increasingly detected along with depletion of oxygen. *Pseudomonas* (bin 16),
 392 *Rhodospirillum* (bin 15), *Rhodopseudomonas* (bin 20), *Rhodobacter* (bin 21), *Dickeya*
 393 (bin 22) and *Enterococcus* (bin 23), belong to the phylum of *Proteobacteria*, had the
 394 intrinsic property of expressing the Fe-only nitrogenases to produce an amount of CH₄

and NH_3 with light and CO_2 and N_2 (Fixen et al., 2016). This indicated the phenomenon of CH_4 production and N_2 fixation in the presence of oxygen at the early stage of culturing. *Rhodospirillum*, *Rhodobacter*, and *Azotobacter* were detected, and each can produce CH_4 when grown with using N_2 as a sole nitrogen source under Fe-only nitrogenase expressing condition (Zhou et al., 2018; Karmann et al., 2019). As one of potential reductant for nitrogenase, 41.44 μM of hydrogen was detected at 4th day, then dropping extensively to 9.51 μM at 17th day, and eventually to 0.57 μM at 53rd day (Fig. 1a). Metagenomic analysis for hydrogenase and hydrogenlyase genes showed *Clostridium* (bin 8) and *Vibrio* (bin 11) were dominant in samples (Fig. S8). Such two genus were capable of hydrogen generation (Diender et al., 2015).

In summary, the bioconversion pathway from CO_2 to the gaseous hydrocarbons in this study was proposed as shown in Fig. 6 that CO_2 fixation was started with CBB cycle, then transferred to rTCA cycle and WL pathway to produce the precursors and energy for further process. Subsequently, the synthesis of C_1 - C_6 hydrocarbons were dominantly catalyzed by nitrogenases. Particularly, the synthesis of methane, observed at the beginning of culturing, was aerobically proceeded by nitrogenase. It was conformed with the previous description.

3.5. Artificial consortium for CO_2 conversion

56 strains were obtained and identified by 16S rRNA sequencing. Among of them, some facultatively and obligately anaerobic strains shown in Table S3 were purposely selected for the reconstruction of a consortium to tentatively validate the pathway of the CO_2 reduction to the gaseous hydrocarbons under the original condition, based on

the above analysis of the 16S rRNA and metagenomic sequencing. The strains, including *Pseudomonas* sp., *Serratia* sp., *Candidatus* sp., *Clostridium* sp., *Sporomusa* sp., *Salmonella* sp., *Rhodospirillum* sp., *Thalassospira* sp., *Thioclava* sp., *Stenotrophomonas* sp. and *Desulfovibrio* sp., which had the relative higher abundance in the enriched community, were selected to construct the new consortium. Importantly, their own functions of completing CBB, rTCA, WL pathway and encoding AD and nitrogenases were essential to such bioconversion. The optimization of the combination was conducted (Fig. S9) As GC data showed in Fig. 7, the optimized consortium also showed the significant ability of CO₂ reduction to the gaseous hydrocarbons. The maximum yield of total gaseous hydrocarbons was 42.97 uM observed at 7th day. The average reduction rate of CO₂ was 107.54 uM/day by decreasing from 5617.89 uM to 241.01 uM during 50 days culturing, which was higher than the rate of the original consortium shown in Fig. 1a. Nitrogen was fixed at the rapid decreasing rate of 299.01 uM/day in first three days, which was also higher than that of the original consortium (161.93 uM/day). It indicated that the reconstructed consortium had the more intensive activities of nitrogenase than. 12.69 uM of hydrogen was detected at 3rd day, then obviously dropped to 0.51 uM at 7th day and eventually to 0.07 uM at 50rd day. This phenomenon demonstrated the significant intensity of nitrogenase catalysis (Hoffman et al., 2014). Compared with the original consortium, the obvious difference was observed that all gaseous hydrocarbons were simultaneously produced by the optimized consortium, and the majority of them reached the highest production rate in early three days. n-Butane and n-pentane were dominant hydrocarbon in the mixture, the maximum

value was 10.62 uM at 18th day for n-butane and 12.75 uM at 7 days n-pentane respectively (Fig. 7b). The original profiles of gas chromatography from the reconstructed consortium were presented in Fig. S10.

For the controls with no light, the maximum yield of total gaseous hydrocarbons was 0.37 uM observed at 43th day (Fig. S11a), which was obviously lower than the samples with light. CO₂ and nitrogen were slightly fixed. Methane was the dominant composition accounting for more than 60%, the other hydrocarbons were negligibly detected (Fig. S11b). This phenomenon was very similar to the description as the CO/CO₂ reduction by Nitrogenase (Fixen et al., 2016), indicating nitrogenase was mainly responsible for hydrocarbon synthesis.

4. Conclusion

With the purpose of constructing a robust and highly-efficient CO₂ bioconversion platform for the industrial application, this study preferred to enrich the original consortia from the marine, and explored the metabolism networks via 16S rRNA and the metagenomic sequencing to target the key microorganisms and enzymes, according to the construction strategy of the general CBP platform. Great efforts on pure isolation from the enriched consortium were made, and the neural network was applied to optimize the combination with using the selected *Pseudomonas sp.*, *Serratia sp.*, *Candidatus sp.*, *Clostridium sp.*, *Enterococcus sp.*, *Salmonella sp.*, *Rhodospirillum sp.*, *Thalassospira sp.*, *Thioclava sp.*, *Stenotrophomonas sp.* and *Desulfovibrio sp.* Eventually, a heterogeneous consortium was tentatively and artificially constructed, which have demonstrated the significantly impressive performance on CO₂ reduction

to the gaseous alkane and alkenes. The total yield of 42.97 μM , the metabolite diversity ($\text{C}_1\text{-C}_6$ alkanes and $\text{C}_2\text{-C}_3$ alkenes), and CO_2 reduction rate of 107.54 $\mu\text{M/day}$ were obviously higher than that achieved by the pure microorganisms or enzymes which has been extensively reported in previous studies. To some extents, these activities also proved the important role of rTCA and WL pathway on CO_2 fixation, and nitrogenase on hydrocarbons synthesis in our proposed pathway. Particularly, this consortium also showed its performance on CO_2/N_2 fixation and hydrocarbon synthesis with no light that methane was accounted for more than 60% in the mixture of the produced hydrocarbons. This further proved the nitrogenase on hydrocarbons synthesis. The comparison between the original and the reconstructed consortium indicated some specific microorganisms play important role on the efficiency and direction of hydrocarbon synthesis. Therefore, the more efforts on the exploration and identification of the new specific microbes, and metabolic network of the enriched consortium will be continuously made, especially the works on WL path, hydrogen production, and nitrogenase. Following this approach, CBP platform of CO_2 conversion could be rapidly constructed for diverse molecules synthesizing. Through this strategy, a microbial “ CO_2 -hydrogenation or “Fischer-Tropsch process” was suggested and could be potential for industrial application.

Acknowledgments

We thank Prof. William A. Goddard (at California Institute of Technology) for assistance with nitrogenase analysis, and Dr. Shaojin Wang (at Department of microbiology, Nankai University) for assistance with metagenomic analysis; Dr. Jing

Zhao for the assistance of reaction energy analysis and setup of photo-reactor. This study was supported by the National Natural Science Foundation of Tianjin (20JCYBJC01280), China National Key Research and Development Project (2018YFA0902101), and Key science and technology project of Karamay (2019ZD002B).

References

- Adam, P.S., Borrel, G., Gribaldo, S., 2019. An archaeal origin of the Wood-Ljungdahl H4MPT branch and the emergence of bacterial methylotrophy. *Nat. Microbiol.* 4:2155-2163. <http://dx.doi.org/10.1007/BF00425584>.
- Berg, I.A., Kockelkorn, D., Ramos-Vera W.H., Say RF, Zarzycki J, Hügler, M., Alber, B.E., Fuchs, G., 2010. Autotrophic carbon fixation in Archaea. *Nat Rev Microbiol.* 8:447-460. <http://dx.doi.org/10.1038/nrmicro2365>.
- Blanchet, E., Duquenne, F., Rafrafi, Y., Etcheverry, L., Erable, B., Bergel, A., 2015. Importance of the hydrogen route in up-scaling electrosynthesis for microbial CO₂ reduction. *Energy Environ. Sci.* 8:3731-3744.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics.* 30(15):2114-2120. <http://dx.doi.org/10.1093/bioinformatics/btu170>.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al, 2015. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods.* 74:1343-1352. <http://dx.doi.org/10.1038/nmeth.f.303>.

- Chen, P.Y.T., Li, B., Drennan, C.L., Elliott, S.J., 2019. A Reverse TCA Cycle 2-Oxoacid:
Ferredoxin oxidoreductase that makes C-C bonds from CO₂. *Joule*. 3(2):595-611.
- Cho, W., Yu, H., Mo, Y., 2017. CO₂ conversion to chemicals and fuel for carbon
utilization. <http://dx.doi.org/10.5772/67316>.
- Coyte, K.Z., Schluter, J., Foster, K.R., 2015. The ecology of the microbiome: networks,
competition, and stability. *Science*. 350:663-666.
<http://dx.doi.org/10.1126/science.aad2602>.
- DeSantis, T.Z., Hugenholtz, P., Larsen N., Rojas, M., Brodie, E.L., Keller, K., Huber,
T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimerachecked 16S
rRNA gene database and workbench compatible with ARB. *Appl Environ
Microbiol*. 72(7):0695072.
- Diender, M., Stams, A.J.M., Sousa, D.Z., 2015. Pathways and bioenergetics of
anaerobic carbon monoxide fermentation. *Front Microbiol*. 6:1275-1293.
<http://dx.doi.org/10.3389/fmicb.2015.01275>.
- Doukov, T.I., Iverson, T.M., Seravalli, J., Ragsdale, S.W., Drennan, C.L., 2002. A Ni-
Fe-Cu Center in a bifunctional carbon monoxide dehydrogenase/AcetylCoA
synthase. *Science*. 298(5593):567-572.
- Fixena, K.R. Zheng, Y., Harris, D.F., Shaw, S., Yang, Z., Dean, D.R., Seefeldt, L.C.,
Harwood, C.S., 2016. Light-driven carbon dioxide reduction to methane by
nitrogenase in a photosynthetic bacterium. *Proc. Natl. Acad. Sci. U. S. A.* 113,
10163-10167. <http://dx.doi.org/10.1073/pnas.1611043113>.
- Gupta, A., Reizman, I.M.B., Reisch, C.R., Prather, K.L.J., 2017. Dynamic regulation of

- 527 metabolic flux in engineered bacteria using a pathway-independent quorum-
 528 sensing circuit. Nat. Biotechnol. 35:273-279. <http://dx.doi.org/10.1038/nbt.3796>
- 529 Hill, E.A., Chrisler, W.B., Beliaev, A.S., Bernstein, H.C., 2017. A flexible microbial co-
 530 culture platform for simultaneous utilization of methane and carbon dioxide from
 531 gas feedstocks. Bioresour. Technol. 228:250-256.
 532 <http://dx.doi.org/10.1016/j.biortech.2016.12.111>
- 533 Hiras, J., Wu, Y.W., Eichorst, S.A., Simmons, B.A., Singer, S.W., 2016. Refining the
 534 phylum *Chlorobi* by resolving the phylogeny and metabolic potential of the
 535 representative of a deeply branching, uncultivated lineage. ISME J. 10:833-845.
 536 <http://dx.doi.org/10.1038/ismej.2015.158>
- 537 Hoffman, B.M., Lukoyanov, D., Yang, Z., Dean, D.R., Lance C, 2014. Mechanism of
 538 Nitrogen Fixation by Nitrogenase: The Next Stage. Chem. Rev. 114, 8, 4041-4062.
- 539 Igarashi, Y., Kodama, T., 1996. Genes related to carbon dioxide fixation in
 540 *Hydrogenovibrio marinus* and *Pseudomonas hydrogenothermophila*. In: Lidstrom
 541 ME, Tabita FR (eds). Microbial Growth on C₁ Compounds. pp 8893.
- 542 Inoue, K., Nishimura, M., Nayak, B.B., Kogure, K., 2007. Separation of marine bacteria
 543 according to buoyant density by use of the densitydependent cell sorting method.
 544 Appl. Environ. Microbiol. 73(4):1049-1053.
- 545 Javor, B.J., 1988. CO₂ fixation in *Halobacteria*. Arch Microbiol. 149:433-440.
- 546 Jones, S., 2008. Five ways to cycle carbon. Nat. Rev. Microbiol. 6:95.
 547 <http://dx.doi.org/10.1038/nrmicro1847>
- 548 Kallio, P., Pásztor, A., Thiel, K., Akhtar, M. K. & Jones, P. R, 2014. An engineered

- 549 pathway for the biosynthesis of renewable propane. *Nat. Commun.* 5, 4731.
- 550 Karmann, S., Panke, S., Zinn, M., 2019. Fed-Batch Cultivations of *Rhodospirillum*
- 551 *rubrum*. *Front. Bioeng. Biotechnol.* 7:59. doi: 10.3389/fbioe.2019.00059.
- 552 Khara, B., Menon, N., Levy, C., Mansell, D., Das, D., Marsh, E.N.G., Leys, D., Scrutton,
- 553 N.S., 2013. Production of propane and other short-chain alkanes by structure-
- 554 based engineering of ligand specificity in aldehyde deformylating oxygenase.
- 555 *Chembiochem.* 14:1204-1209. <http://dx.doi.org/10.1002/cbic.201300307>
- 556 Kintisch, E., 2015. After Paris: the rocky road ahead. *Science.* 350:1018-1019.
- 557 <http://dx.doi.org/10.1126/science.350.6264.1018>
- 558 Kondratenko, E.V., Mul, G., Baltrusaitis, Larrazábal, G.O., Pérez-Ramírez, J., 2013.
- 559 Status and perspectives of CO₂ conversion into fuels and chemicals by catalytic,
- 560 photocatalytic and electrocatalytic processes. *Energy Environ. Sci.* 6, 3112-3135.
- 561 <http://dx.doi.org/10.1039/c3ee41272e>.
- 562 Kumar, M., Gazara, R.K., Verma, S., Kumar, K., Verma, P.K., Thakur, I.S., 2016.
- 563 Genome sequence of carbon dioxide sequestering *Serratia sp* strain ISTD04
- 564 isolated from marble mining rocks. *Genome. Announc.* 4(5):e01141.
- 565 <http://dx.doi.org/10.1128/genomeA.01141-16>.
- 566 Lee, C.C., Hu, Y., Ribbe, M.W., 2018. Reduction and condensation of aldehydes by the
- 567 isolated cofactor of nitrogenase. *ACS Cent. Sci.* 4, 1430-1435.
- 568 Liao, J.C., Mi, L., Pontrelli, S., Luo, S., 2016. Fuelling the future: Microbial
- 569 engineering for the production of sustainable biofuels. *Nat. Rev. Microbiol.*
- 570 14:288-304. <http://dx.doi.org/10.1038/nrmicro.2016.32>

- 571 Liu, X., Miao, R., Lindberg, P., Lindblad, P., 2019. Modular engineering for efficient
572 photosynthetic biosynthesis of 1-butanol from CO₂ in cyanobacteria. *Energy*
573 *Environ Sci.*12:2765-2777. <http://dx.doi.org/10.1039/C9EE01214A>
- 574 Liu, Y., Ding, M., Ling, W., Yang, Y., Zhou, X., Li, B.Z., et al, 2017. A three-species
575 microbial consortium for power generation. *Energy. Environ. Sci.*10:1600-1609.
576 <http://dx.doi.org/10.1039/C6EE03705D>
- 577 Li, D, Liu, C.M., Luo, R., Sadakane, K., Lam, T.W, 2015. MEGAHIT: an ultrafast
578 singlenode solution for large and complex metagenomics assembly via succinct de
579 Bruijn graph. *Bioinformatics.* 31:1674-1676.
580 <http://dx.doi.org/10.1093/bioinformatics/btv033>
- 581 Ni, J., Liu, H.Y., Tao, F., Wu, Y.T., Xu, P., 2018. Remodeling of the photosynthetic chain
582 promotes direct CO₂ conversion to valuable aromatics. *Angew. Chemie. Int. Ed.*
583 57:15990-15994.
- 584 Otto, A., Grube, T., Schiebah, S., Stolten, D., 2015. Closing the loop: captured CO₂ as
585 a feedstock in the chemical industry. *Energy. Environ. Sci.* 8: 3283-3297.
586 <http://dx.doi.org/10.1039/C5EE02591E>.
- 587 Park, M.O., 2005. New pathway for long-chain n-alkane synthesis via 1alcohol in
588 *Vibrio furnissii* M1. *J. Bacteriol.* 187:1426-1429.
- 589 Patro, R., Dugga, G., Love, M.I., Irizarry, R.A., Kingsford, C., 2017. Salmon provides
590 fast and biasaware quantification of transcript expression. *Nat. Methods.* 14:417-
591 419.
- 592 Pérez-Etayo, L., de Miguel, M.J., Conde-Álvarez, R., Muñoz, P.M., Khames, P.M.,

- Iriarte, M., Moriyón, I., Zúñiga-Ripa, A., 2018. The CO₂-dependence of *Brucella ovis* and *Brucella abortus biovars* is caused by defective carbonic anhydrases. *Vet Res.* 49:85-97. <http://dx.doi.org/10.1186/s13567-018-0583-1>
- Rashid, N., Saif Ur Rehman, M., Sadiq, M., Mahmood, T., Han, J., 2014. Current status, issues and developments in microalgae derived biodiesel production. *Renew. Sustain. Energy. Rev.* 40:760-778.
- Rasigraf, O., Kool, D.M., Jetten, M.S.M., Sinninghe Damsté, J.S., Ettwig, K.F., 2014. Autotrophic carbon dioxide fixation via the Calvin-Benson-Bassham Cycle by the denitrifying methanotroph *Candidatus Methyloirabilis oxyfera*. *Appl. Environ. Microbiol.* 80: 2451-2460. <http://dx.doi.org/10.1128/AEM.04199-13>
- Schirmer, A., Rude, M.A., Li, X., Popova, E., Del Cardayre, S.B., 2010. Microbial biosynthesis of alkanes. *Science.* 329(5991):559-562. <http://dx.doi.org/10.1126/science.1187936>
- Scrutton, N.S., 2017. Enzymes make light work of hydrocarbon production. *Science.* 357:872-873. <http://dx.doi.org/10.1126/science.aao4399>
- Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics.* 30(14):2068-2069. <http://dx.doi.org/10.1093/bioinformatics/btu153>.
- Shahab, R.L., Brethauer, S., Davey, M.P., Smith, A.G., Vignolini, S., Luterbacher, J.S., Studer, M.H., 2020. A heterogeneous microbial consortium producing short-chain fatty acids from lignocellulose. *Science.* 369:6507, eabb1214.
- Shimamura, T., Watanabe, S., Sasaki, S., 1985. Enhancement of enterotoxin production by carbon dioxide in *Vibrio cholerae*. *Infect. Immun.* 49:455-460.

<http://dx.doi.org/10.1128/iai.49.2.455-456.1985>

Spurgeon, J.M., Kumar, B., 2018. A comparative technoeconomic analysis of pathways for commercial electrochemical CO₂ reduction to liquid products. *Energy. Environ. Sci.* 11:1536-1551.

Stephens, K., Pozo, M., Tsao, C., Hauk, P., Bentley, W.E., 2019. Bacterial co-culture with cell signaling translator and growth controller modules for autonomously regulated culture composition. *Nat. Commun.* 10:4129-4140. <http://dx.doi.org/10.1038/s41467-019-12027-6>

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M., 2014. Development of a prokaryotic universal primer for simultaneous analysis of *Bacteria* and *Archaea* using next-generation sequencing. *PLoS One.* 9(8):e105592. <http://dx.doi.org/10.1371/journal.pone.0105592>

Takai, K., Campbell, B.J., Cary, S.C., Suzuki, M., Oida, H., Nunoura, T., Hirayama, H., Nakagawa, S., Suzuki, Y., Inagaki, F., 2005. Enzymatic and genetic characterization of carbon and energy metabolisms by deep-sea hydrothermal chemolithoautotrophic isolates of *Epsilonproteobacteria*. *Appl Environ Microbiol.* 71:7310-7320. <http://dx.doi.org/10.1128/AEM.71.11.7310-7320.2005>

Tsoi, R., Wu, F., Zhang, C., Bewick, S., Karig, D., You, L., 2018. Metabolic division of labor in microbial systems. *Proc. Natl. Acad. Sci. U.S.A.* 115:2526-2531.

Yu, S., Jain, P.K., 2019. Plasmonic photosynthesis of C₁-C₃ hydrocarbons from carbon dioxide assisted by an ionic liquid. *Nat. Commun.* 10:2022-2029. <http://dx.doi.org/10.1038/s41467-019-10084-5>

- 637 Wu, Y.W., Tang, Y.H., Tringe, S.G., 2014. Simmons BA, Singer SW. MaxBin: an
638 automated binning method to recover individual genomes from metagenomes
639 using an expectation-maximization algorithm. *Microbiome*. 2:2644.
640 <http://dx.doi.org/10.1186/2049-2618-2-26>
- 641 Zhang, Y., Wei, W., Wang, Y., Ni, B., 2021. Enhancing methane production from algae
642 anaerobic digestion using diatomite. *J. Clean. Prod.* 315, 128138.
- 643 Zhou, Y., Grice, K., Stuart-Williams, H., Farquhar, G.D., Hocart, C.H., Lu, H., Liu, W.,
644 2010. Biosynthetic origin of the sawtoothed profile in $\delta^{13}\text{C}$ and $\delta^2\text{H}$ of nalkanes
645 and systematic isotopic differences between n, iso and anteisoalkanes in leaf
646 waxes of land plants. *Phytochemistry*. 71:388-403.
- 647 Zhou, Y.J., Kerkhoven, E.J., Nielsen, J., 2018. Barriers and opportunities in biobased
648 production of hydrocarbons. *Nat. Energy*. 3:925-935.
- 649

Figures captions

Fig. 1. Conversion of carbon dioxide to short-chain hydrocarbons by long-term cultivation of the enriched microbial consortia. (A) Detection of total synthesized hydrocarbons, oxygen, dinitrogen, carbon dioxide, and hydrogen; (B) C1-C6 alkanes or alkenes were detected through the culturing, when carbon dioxide as solo carbon source with exposed to LED light.

Fig. 2. DNA samples for community analysis were obtained for all data points. Taxonomic composition of the enriching microbial communities in cultures. Each OTU with an average normalized abundance of $\geq 1\%$ of the community under any treatment is labeled according to the lowest taxonomic rank assigned to them. Based on 16S rRNA, bacterial and archaea OUTs in various levels. Each dataset represents the average of independently sequenced triplicate cultures.

Fig. 3. Functional overview of high-quality binned genomes. The size of the filled circle indicates the presence of the metabolic activities in the corresponding genome from each sample, according to COG (Unicellular orthologs) and KOG (Eukaryote orthologs). A metabolism was considered present only if all genes encoding at least one full enzymatic pathway capable of carrying out that metabolic process were found in the genome. A bioenergetic complex was considered present only if all genes encoding the necessary subunits form a functional protein complex were found in the genome.

Fig. 4. Accumulated gene abundance for the completed pathways of carbon dioxide fixation. Calvin-Benson cycle (M00165), Reductive citrate cycle (M00173), and WL pathway (M00377) from all samples.

Fig. 5. Comparison of accumulated gene abundance of hydrocarbon synthesis. a) among the nitrogenase, and carboxylic acid reductase, aldehyde decarbonylase, and b) within three types of nitrogenases.

Fig. 6. Schematic pathway for our bioconversion process from carbon dioxide to short chain hydrocarbons. Based on the analysis of metabolites, 16S rRNA sequencing, and metagenomic sequencing. (A) represents Calvin-Benson cycle. (B) represents reductive citrate cycle. (C) represents WL pathway. (D) represents the pathway of hydrocarbon synthesis with ADO and CAR, and (E) represents the pathway of hydrocarbon synthesis with nitrogenase.

Fig. 7. Conversion of carbon dioxide to short-chain hydrocarbons by the established heterogeneous consortium. (A) Detection of oxygen, dinitrogen, carbon dioxide, and hydrogen; (B) C₁-C₆ alkanes or alkenes were detected through the culturing, when carbon dioxide as solo carbon source with exposed to LED light.

A

B

Bacteria

Archaea

Journal Pre-proof

Phylum

Proteobacteria
Firmicutes
Actinobacteria
Synergistetes
Cyanobacteria
Gemmatimonadetes
OP9
WS6

Fusobacteria
Bacteroidetes
Spirochaetes
Chlorobi
Verrucomicrobia
Chloroflexi
Thermi
Tenericutes

Euryarchaeota
Crenarchaeota

Class

Gammaproteobacteria
Alphaproteobacteria
Deltaproteobacteria
Bacteroidia
Sphingobacteriia
Actinobacteria
Spirochaetes
Ignavibacteria
Cytophagia
Verrucomicrobiae

Fusobacteriia
Clostridia
Epsilonproteobacteria
Betaproteobacteria
Flavobacteriia
Bacilli
Synergistia
Symploetrichi
Chloroplast
other

Methanomicrobia
MCG
Methanobacteria
Thaumarchaeota
MBGB
MBGA
Thermoplasmata

Order

Enterobacteriales
Alteromonadales
Rhodobacterales
Desulfovibrionales
Oceanospirillales
Burkholderiales
Sphingobacteriales
Rhizobiales
Actinomycetales
Spirochaetales

Pseudomonadales
Fusobacteriales
Clostridiales
Campylobacterales
Bacteroidales
Xanthomonadales
Vibrionales
Flavobacteriales
Lactobacillales
other

Methanosarcinales
pGrFC26
Methanomicrobiales
Methanobacteriales
Cenarchaeales
YC-E6
B10
E2
Nitrososphaerales

Family

Enterobacteriaceae
Alteromonadaceae
Rhodobacteraceae
Campylobacteraceae
Tissierellaceae
S24-7
Sphingobacteriaceae
Clostridiaceae
Flavobacteriaceae
Moraxellaceae

Pseudomonadaceae
Fusobacteriaceae
Desulfovibrionaceae
Oceanospirillaceae
Xanthomonadaceae
Comamonadaceae
Alcaligenaceae
Bacteroidaceae
Lachnospiraceae
other

Methanosarcinaceae
Methanosetaeaceae
Methanospirillaceae
WSA2
Cenarchaeaceae
Methanobacteriaceae
Methanoregulaceae
Methanomicrobiaceae
Marine group II
Methanomassiliococcaceae
Nitrososphaeraeae

Genus

Pseudomonas
Marinobacter
Arcobacter
Marinobacterium
Lysobacter
Clostridium
Vibrio
Marinomonas
Cellvibrio
Alteromonas

Serratia
Propionigenium
Sedimentibacter
Comamonas
Bacteroides
Lactobacillus
Treponema
Thalassospira
Psychrobacter
other

Methanosarcina
Methanoseta
Methanospirillum
Methanobacterium
Nitrosopumilus
Methanolinea
Methanoculleus
Methanomassiliococcus
Methanobrevibacter
Candidatus Nitrososphaera
Methanosphaera
Candidatus Methanoregula
Methanomethylivorans

Species

Serratia marcescens
Vibrio shilonii
Bacteroides acidifaciens
Marinobacter hydrocarbonoclasticus
Actinomadura vinacea
Lactobacillus reuteri
Akkermansia muciniphila
Ruminococcus gnavus
Bacillus humi
Desulfovibrio aminophilus
Alcanivorax dieselolei
Brachybacterium conglomeratum
Shewanella algae
Luteibacter rhizovicinus
Blautia producta

Methanosarcina mazei
Methanobacterium bryantii

A

normalized gene number

B

normalized gene abundance (TPM)

A

B

Highlights

- Photo-driven and direct CO₂ reduction to hydrocarbons by marine microorganism.
- Short chain alkanes (C₁-C₆) and alkenes (C₂-C₃) were biosynthesized.
- CO₂ reduction rate was 100.87 $\mu\text{M day}^{-1}$.
- CBB cycle, reductive TCA cycle and Wood-Ljungdahl pathway were responsible for carbon fixation.
- Nitrogenase played important roles on hydrocarbon synthesis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.