CO_2 FIXATION AS METHANE, ORGANIC ACID, OR AMORPHOUS CARBON: NEW INSIGHTS INTO THE CARBON SEQUESTRATION POTENTIAL OF ANAEROBIC DIGESTION

Tengyu Zhang[1,2], Jingxin Zhang*[1]

1. China-UK Low Carbon College, Shanghai Jiao Tong University, Shanghai 200240, China

2. *School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China*

**E-mail: lcczjx@sjtu.edu.cn*

ABSTRACT

Anaerobic digestion (AD) is a usually technology to facilitating carbon fixcation by conversion from complex organic matter to volatile fatty acids and CH_4, however, the problem of CO_2 emission has not been solved with AD. Amorphous carbon formation be found as a more directly way to fix carbon in AD. This study aimed to elucidate how the amorphous carbon can be formed by organic matter or CO_2 by anaerobic microorganisms. As result, the amorphous carbon was found in AD of inorganic and mix carbon source with yields of 0.38 μg/10^5cell and 3 μg/10^5cell, and the characteristics were characterized by Raman microscopy. Isotope labeling revealed that the fixation of CO_2 to amorphous carbon is mainly dependent on the reversed oxidative tricarboxylic acid cycle (roTCA) and hydroxycaproate. Differential pulse voltammetry combined with gene abundance analysis showed that the flavins electron bifurcation (EB) are involved in the electron transfer. The microbial isothermal calorimeter further tested the metabolic calorific value, indicating that anaerobic microorganisms can carry out autotrophic fixation of CO_2 under the energy support of EB. The results of metagenome support the large REDOX equivalents of the EB input to support the roTCA cycle. This research is instrumental in comprehending the mechanism of CO_2 fixed in solid carbon in anaerobic environment. Additionally, there is a new understanding of the possibility of developing carbon-negative technologies in anaerobic biological treatment.

KEY WORDS

anaerobic digestion, CO_2 fixation, amorphous carbon, electronic bifurcation, electron transport.

1. INTRODUCTION

Anaerobic digestion (AD) is a low-carbon biological treatment technology[1]. However, the discovery of amorphous carbon means that AD still has research gaps. Allen found that anaerobic organisms contribute to the formation of amorphous carbon directly from CO_2[2]. However, the discovery of amorphous carbon means that AD still has a research gap. Generally, inorganic carbon fixation is facilitated through various autotrophic pathways, including the reversed oxidative tricarboxylic acid cycle (roTCA). Given the inherent drive of microorganisms towards optimizing energy and matter utilization, the conversion of CO_2 to organic carbon can proceed spontaneously. However, the production of solid inorganic carbon has no obvious significance for microbial energy storage, although the reaction can also be spontaneous[3]. The transition from CO_2 to graphite possessing an entropy of 5.74 J/k·mol, contrary to the law of entropy increase. Which underscores the significant activation energy required for this reaction is far from negligible. In general, the roTCA cycle and electron bifurcation (EB) are considered in the process of transforming gaseous CO_2 into solid form[4].

This study intends to research the CO_2 fixation to amorphous carbon process in AD. We employed a microorganism solution system to mimic the fixation of CO_2 under AD to: (1) research the novel CO_2 fixation way and figure out the amorphous carbon metabolic pathways in AD; and (2) the first time to explore the energy support of EB in the formation of amorphous carbon.

2.MATERIALS AND METHODS

2.1 Experimental designs

The microbial solution from sludge was put into 80 ml test tubes for culture. CO_2 was injected into the tubes for two ways (Circulate:1L/min for 1min; Seal: 10ml CO_2/50ml microbial solution for 1atm).

2.2 Extraction and analysis of black materials containing amorphous carbon

Raman spectroscopy is used to analyze the obtained part of the black microsphere to determine the characteristic peak of elemental carbon, the XPS to determine the valence band, and the 13 C abundance of carbon dioxide after combustion with oxygen.

2.3 Microbial isothermal calorimetry

The 1M (saturated concentration) DIC was configured with PBS and the concentrated sample was washed and suspended with PBS. The 500μl sample was added to the reactor, and the temperature of the detection chamber was set at a constant 35℃. The change of heat value in the process of reaction was measured.

2.4 The other Analysis Method

Gas chromatography GC-2014C (Shimadzu, Japan) was used to analyze the biogas content. electron carrier concentration, DPV determination of flavin activity were performed in an electrochemical workstation.

3. RESULTS AND DISCUSSION

3.1 Discovery of amorphous carbon in anaerobic microorganisms

The extraction of amorphous carbon was carried out after one month culture. And Raman spectrum represents the degree of disorder of amorphous carbon (Fig.1a, b). The XPS result showed that This disordered structure has both sp2 and sp3 hybrid orbitals. According to the amount of amorphous carbon obtained in the CO_2 only reactor, it seems inevitable that CO_2 and biomass will eventually move to amorphous carbon even if no additional electron donor is added over a long period of operation.

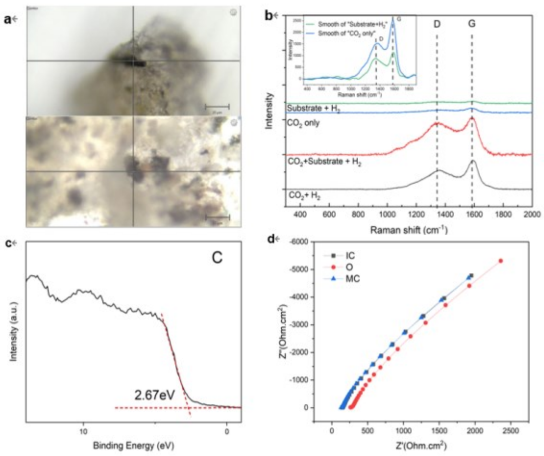

Fig.l: Characterization of amorphous carbon. Optical microscopy (a) and Raman spectroscopy(b). Detection of amorphous carbon price bands with XPS (c) EIS detection of microbial systems(d).

3.2 3.3 REDOX activity of Flavin-based electron bifurcation during CO_2 fixation

The results of electrochemical activity of FAD favors inorganic carbon utilization by anaerobic microorganisms (Fig.2). The inorganic carbon source group (IC) exhibited excellent FAD-reduction and oxidation characteristics at -580 mv and -424 mv. The microorganisms cultured with inorganic carbon source showed higher FAD REDOX activity, while weaker ascorbic acid oxidation activity.

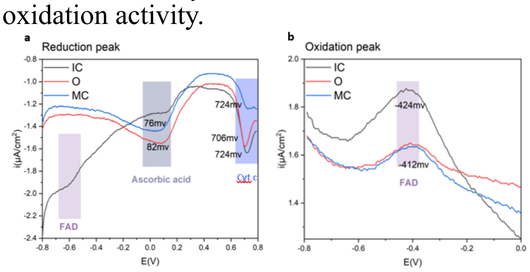

Fig.2: DPV test for flavins and ascorbic acid. Reduction peak (a) and oxidation peak (b). Group O: glucose only as substrate, group IC: CO_2 only as carbon source, group MC: glucose and CO_2 as mixed carbon source.

3.3 Thermodynamic conditions support by electron bifurcation

The biological process of fixing carbon dioxide as a solid carbon requires high energy metabolism within the cell. The results showed why microbial metabolism with EB can support this carbon fixcation (Fig.3). The actual reaction calorific in 500μl cell sample is the integral area multiplied by time to obtain that the microbial without carbon source substrate is 0.035J. The reaction calorific value of inorganic carbon source is 0.143J at 37℃, and the actual reaction calorific value of microbial and inorganic carbon source is 0.521J.

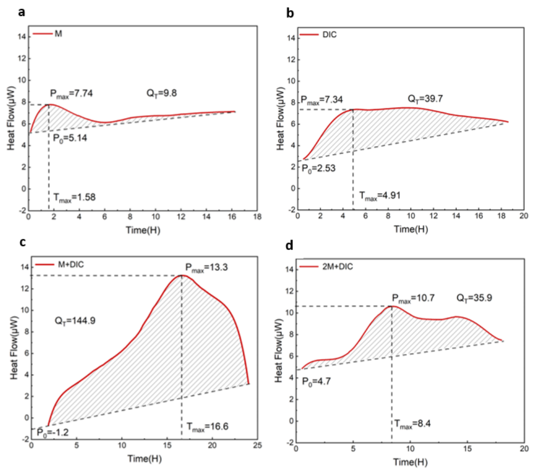

Fig.3: Microbial heat change in the CO_2 conversion process. Microbial only (a), DIC: Soluble inorganic carbon+inactivated microorganism (b), microorganism+DIC: Microorganism and soluble inorganic carbon (c), double microorganism+DIC(d).

ACKNOWLEDGEMENTS

This project was supported by the National Natural Science Foundation of China (52270131).

REFERENCES

1 Zhang, T., Zhang, J., Zhang, P., Wang, J. and He, Y., 2023. Electronic Bifurcation: A New Perspective on Fe Bio-Utilization in Anaerobic Digestion of Lactate. Environ. Sci. Technol 57(28), 10448-10457.

2 Allen, K.D., Wegener, G., Matthew Sublett, D., Bodnar, R.J., Feng, X., Wendt, J. and White, R.H., 2021. Biogenic formation of amorphous carbon by anaerobic methanotrophs and selects methanogens. *Sci. Adv.* 7(44), eabg9739.

3 Ragsdale, S.W., 2018. Stealth reactions driving carbon fixation. Science 359(6375), 517-518.

4 Steffens, L., Pettinato, E., Steiner, T.M., Mall, A., König, S., Eisenreich, W. and Berg, I.A., 2021. High CO_2 levels drive the TCA cycle backwards towards autotrophy. Nature 592(7856), 784-788.