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Severe cyanobacteria accumulation potentially induces methylotrophic methane producing pathway in eutrophic lakes☆

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ABSTRACT

Although cyanobacteria blooms lead to an increase in methane (CH_4) emissions in eutrophic lakes have been intensively studied, the methane production pathways and driving mechanisms of the associated CH_4 emissions are still unclear. In this study, the hypereutrophic Lake Taihu, which has extreme cyanobacteria accumulation, was selected to test hypothesis of a potential methylotrophic CH_4 production pathway. Field observation displayed that the CH_4 emission flux from the area with cyanobacteria accumulation was 867.01 μg m^{-2}·min^{-1}, much higher than the flux of 3.44 μg m^{-2}·min^{-1} in the non-cyanobacteria accumulation area. The corresponding abundance of methane-producing archaea (MPA) in the cyanobacteria-concentrated area was 77.33% higher than that in the non-concentrated area via RT-qPCR technologies. Synchronously, sediments from these areas were incubated in anaerobic bottles, and results exhibited the high CH_4 emission potential of the cyanobacteria concentrated area versus the non-concentrated area (1199.26 vs. 205.76 μmol/L) and more active biological processes (CO_2 emission, 2072.8 vs. −714.62 μmol/L). We also found evidence for the methylotrophic methane producing pathway, which contributed to the high CH_4 emission flux from the cyanobacteria accumulation area. Firstly, cyanobacteria decomposition provided the prerequisite of abundant methyl thioether substances, including DMS, DMDS, and DMTS. Results showed that the content of methyl thioethers increased with the biomass of cyanobacteria, and the released DMS, DMDS, and DMTS was up to 96.35, 3.22 and 13.61 μg/L, respectively, in the highly concentrated 25000 g/cm^3 cyanobacteria treatment. Then, cyanobacteria decomposition created anaerobic microenvironments (DO 0.06 mg/L and Eh −304.8Mv) for methylotrophic methane production. Lastly, the relative abundance of Methanosarcinales was increased from 7.67% at the initial stage to 36.02% at the final stage within a sediment treatment with 10 mmol/L $N(CH_3)_3$. Quantitatively, the proportion of the methylotrophic methane production pathway was as high as 32.58%. This finding is crucial for accurately evaluating the methane emission flux, and evaluating future management strategies of eutrophic lakes.

1. Introduction

Lakes are important natural sources of CH_4, accounting for 16%–24% of the global natural CH_4 emissions (Zhou et al., 2019; Saunois et al., 2020). In recent decades, due to humans' large-scale land use and artificial changes in the nutrient cycle, a large amount of nitrogen (N) and phosphorous (P) has been discharged into freshwater lakes, thus triggering outbreaks of cyanobacteria blooms (Carey et al., 2012). It has been reported that there is a bidirectional feedback between cyanobacteria blooms, climate warming and lake eutrophication (Davis et al., 2009; Yan et al., 2017). Rising temperature enhances the growth rates of cyanobacteria blooms by increasing the stability of the water column and lengthening the duration of thermal stratification, which is favorable for buoyant cyanobacteria (Davis et al., 2009; Mehnert et al., 2010). Simultaneously, elevated CO_2 concentrations lead to increased photosynthesis, since free CO_2 is converted to highly available inorganic

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carbon, including CO_3^{2-} and HCO_3^- (Wetzel, 2001). Furthermore, cyanobacteria-derived organic carbon is mineralized and releases nutrients and CH_4 after the bloom collapses, thus sustaining eutrophication and contributing to climate warming (Bartosiewicz et al., 2021). Therefore, it is crucial to ascertain the impact of cyanobacteria blooms on the CH_4 production processes and mechanisms for accurately evaluating CH_4 emission fluxes and understanding carbon cycle processes in freshwater lakes.

There are three pathways for methane production, namely hydrogenotrophic, acetoclastic, and methylotrophic pathway. In freshwater lakes, it is generally acknowledged that acetoclastic and hydrogenotrophic pathway are the main types of methanogenic reactions due to the low content of methyl compounds (Preheim et al., 2016). Hydrogenotrophic pathway is widely found in nature. Except for a few methane-producing archaea (MPA) that do not perform hydrogenotrophic, most MPA can use the hydrogenotrophic pathway to produce CH_4 (Thauer et al., 2008). In this pathway, most MPA use H_2 as an electron donor and transfer electrons to reduce CO_2 under the action of hydrogenase. In addition, most MPA also can use HCOOH as an electron donor to reduce CO_2. Formate dehydrogenase (FDH) and reducing hydrogenase are used to convert four molecules of HCOOH into $4CO_2$ and $4H_2$, which are then used to produce CH_4 (Wood et al., 2003). In nature, 70% of biogenic CH_4 production comes from the acetoclastic pathway, which produces CH_4 by splitting acetic acid and reducing methyl carbon. Meanwhile, the carboxyl carbon is oxidized to produce CO_2 (Liu et al., 2008). The main reasons for the differences between methane-producing pathways include the substrate concentration, temperature, and pH (Yvon-Durocher et al., 2014; Kelley et al., 2014), with the content of the substrate being one of the most important factors (Kelley et al., 2014). A study found that when the concentration of acetic acid in freshwater sediment is lower than 21.5 μmol/L, even under optimal pH conditions, methanogens cannot undergo normal growth and exert their normal function, and thus the effect of temperature on methane production through acetic acid fermentation is smaller (George et al., 2020).

With cyanobacterial blooms outbreaks, cyanobacteria residue on the surface sediment forms “Cyanobacterial detritus mat” (Qi et al., 2020). The composition of this algal residue is complex and is a significant source of organic matter to the surface sediment. Studies from the eutrophic Chaohu lake and Dianchi lake show that this algal residue causes continuous changes in the sediment composition and activity, and thus creates an unstable matrix for the methanogenesis process (Spooner et al., 2009; Shen et al., 2018). The biodegradable components, which mainly consist of aromatic proteins and soluble microbial metabolites, provide substrate for microbial metabolism, and create an anaerobic environment for the methanogens metabolic processes (Fan et al., 2014; Yang et al., 2021). Meanwhile, due to the shielding effect of cyanobacteria, reoxygenation through the water-air interface is prevented and the decline of cyanobacteria is accelerated (Xing et al., 2011). Cyanobacteria residue settles on the surface of the sediment and decomposes via anaerobic fermentation. This decomposition releases C, N, and P into the sediment and overlying water, which changes the microbial community structure, promotes the activity of microorganisms such as methanogens and stimulates growth (Li et al., 2020). In addition, this process results in organic carbon mineralization in the sediment, which promotes CH_4 emissions (Kelley et al., 1990; Tranvik et al., 2009). CH_4 released from lakes into the atmosphere is the final result of methanogenic processes, and thus methanogenic archaea are one of the microorganisms that play an important role in the CH_4 emission process (Roland et al., 2017). Although much research shows that the growth of cyanobacteria increases CH_4 emissions, little research has been conducted on the effect of severe cyanobacteria accumulation on methane production pathways in different substrates (Yan et al., 2017).

The methylotrophic methane-producing pathway was known to be widespread in marine ecosystems (Zhuang et al., 2018; Xu et al., 2020), but it was generally thought that such a pathway did not occur in freshwater lakes (Whalen et al., 2005; Parkes et al., 2007). However, in some polar and plateau salt lakes, such as Soda Lake in the Arctic and Lake Bangkog Co on the Tibetan Plateau, methylotrophic methane-producing pathways have been found to be an important source of CH_4 production (Deng et al., 2017; Liu et al., 2017). Sulfate reduction and methanogenesis compete for hydrogenotrophic and acetoclastic, while methylotrophic only participate in methanogenesis (Mitterer et al., 2001; Dowrick et al., 2006). A large number of methyl compounds are released during the decomposition process of cyanobacteria, with methyl sulfide substances being important components of cyanobacteria, including dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) (Huang et al., 2020). Studies have shown that the presence of methyl compounds can promote CH_4 production in methylotrophic methane-producing pathways (Penger et al., 2012). Methanosarcinales, which can utilize methanol and methylamine, have been found in many lake sediments, and substrate is one of the most important factors affecting its distribution (Zhu et al., 2012; Lyautey et al., 2021). The long-term existence of cyanobacteria deposits would provide sufficient substrate for methane production from sediments and affect the metabolic cycle of methane, however, the influence of cyanobacteria blooms on methane metabolic cycle is still not clear (Gelesh et al., 2016). Although cyanobacteria accumulation can lead to an increase in CH_4 emissions, few studies have focused on the possible changes to methane production pathways caused by severe cyanobacteria accumulation (Yan et al., 2017). We, therefore, hypothesized that the production of methyl compounds during the decomposition of cyanobacteria could induce methylotrophic methane-producing pathways in eutrophic lakes by providing sufficient substrates for these pathways.

In this study, the hypereutrophic Lake Taihu was selected in consideration of its heavy cyanobacteria accumulation. The CH_4 emission flux and the methanogenic bacteria community composition and abundance were studied in regions of the lake with and without cyanobacteria blooms. In addition, a simulation microcosm was established to explore the release capacity of methyl thioether substances, CH_4 production potential, and substrate sources during the process of cyanobacteria decay. We hypothesized that severe cyanobacterial accumulation may induce methylotrophic CH_4 producing pathways in eutrophic lakes. The findings from this study provide an accurate assessment of CH_4 emissions from cyanobacteria accumulation areas in eutrophic lakes.

2. Materials and methods

2.1. Study site and sample collection

In this study, a cyanobacteria accumulation area and non-cyanobacteria accumulation area (the open lake area) of Taihu Lake were selected to compare the influence of cyanobacterial accumulation on the CH_4 producing pathway. The cyanobacteria accumulation area, located in the Zhushan Bay (31°24′45″N, 120°0′42″E), is surrounded by long strips of dense reed and other emergent plants. Owing to the downwind direction of the prevailing wind in summer, a large amount of cyanobacteria is trapped by the emergent plants in the nearshore with extremely heavy cyanobacteria accumulation (Zhao et al., 2019; Ma et al., 2020), which generally occurs in most of eutrophic lakes worldwide (Xing et al., 2011; Ho et al., 2019). The open area of the lake is away from the cyanobacteria accumulation area (31°24′40″N, 120°1′3″E). Triplicate overlying water samples (20 cm below the water surface) and surface sediments samples (0–10 cm depth) were collected from cyanobacteria accumulation area and the open lake area. Gas samples were collected via floating static chambers (38.5 cm × 30.5 cm × 18.5 cm) every 10 min for 1 h, which have been previously shown to provide unbiased measurements of water-gas exchange (Cole et al., 2010). Dissolved oxygen (DO) and redox potential (Eh) were measured

in-situ by using YSI ProfessionalPlus (A Xylem, USA). In this study, in order to illustrate that severe cyanobacteria accumulation would induce CH_4 emission via the methylotrophic methane production pathway, the emission fluxes in June were selected, since it could represent the average value during cyanobacteria breakout period and verify that this phenomenon might occur in broad spatial scales.

To build the microcosm system, in June 2020, the lake water was collected at 30 cm below the surface near the shore of Zhushan Bay. The surface sediments from the cyanobacteria accumulation area and open lake area were collected using a Peterson dredger. Cyanobacteria were harvested by a plankton net (250 meshes) from the cyanobacteria accumulation area. All samples were stored in an incubator with ice packs and delivered to the laboratory immediately.

2.2. Microcosm system

2.2.1. Methane-producing potential

The microcosm system to measure the methane-producing potential consisted of 12 anaerobic bottles (diameter 7.5 cm and height 50 cm), with the two sampling areas having six replicates each. 100 g of homogenized surface sediment was separately placed into each anaerobic bottle, and then the lake water was added to submerge the sediment. The headspace in each anaerobic bottle was filled with N_2 gas. All of the anaerobic bottles were placed in a biochemical incubator at a temperature of 25 °C. Gas samples were collected in a time series of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 34, 37, 42, and 47 days.

2.2.2. Methane-producing pathways

The microcosm system for examining the methane-producing pathways consisted of 12 anaerobic bottles (diameter 7.5 cm and height 50 cm) for four different substrate types, with each substrate type having three replicates. 100 g of homogenized surface sediment was separately placed into each anaerobic bottle, and then 200 mL of a solution containing 10 mmol/L of CH_3COONa, HCOONa, and $N(CH_3)_3$ was subsequently added to the three different treatment groups. HCOONa as the primary substrate for hydrogenotrophic CH_4 producing pathway, was used to replace H_2/CO_2, which is not readily available. 200 mL of water was added to the fourth group to act as a control. The headspace in each anaerobic bottle was filled with N_2 gas. All of the anaerobic bottles were placed in a biochemical incubator at a temperature of 25 °C. Gas samples were collected in a time series of 1,2,4, 6, 8,10,12,15,18, and 21 days.

2.2.3. Methyl compounds collection after cyanobacteria decay

The microcosm system used to analyze the methyl compounds produced during the decay of cyanobacteria consisted of columns with a diameter of 20 cm and a height of 2 m. The columns consisted of beakers with their sides wrapped in black plastic to avoid light penetration. Homogenized surface sediment was placed into each column up to 20 cm in height, and then filtered lake water was carefully added into each beaker. 0 g/cm^3, 7500 g/cm^3, or 25000 g/cm^3 of cyanobacteria was added into each column (Zhang et al., 2020), to create three treatment groups. All of the beakers were incubated at 30 °C. Gas samples were collected in a time series of 1, 2, 3, 5, 8, 12, 18, and 26 days.

2.3. Chemical analysis

2.3.1. Determination of TN, TP, Chl-a and TOC concentrations

The overlying water samples were analyzed for total nitrogen (TN), total phosphorus (TP), chlorophyll-a (Chl-a), and total organic carbon (TOC) contents. TN was determined photometrically using a UV–vis spectrophotometer (UV-6100, mapada, China). TP was determined by colorimetry after digestion with $K_2S_2O_8$+NaOH. Samples for Chl-a measurement were filtered through a Mili CA membrane (0.45 μm) under low pressure. Then, filters were frozen and extracted using 10 mL of acetone (95%, AR). The optical densities of the extracts at 630, 645, 663 and 750 nm were determined using a UV–vis spectrophotometer (UV-6100, mapada, China) with 1 cm matched cells. Chl-a was determined using the equations derived by Strickland and Parsons (Strickland et al., 1968). TOC was investigated using a TOC analyzer (Analytik Jena HT1300, Germany). The accuracy of TN, TP, TOC and Chl-a concentrations was up to 0.1 mg/L level.

The sediments samples were freeze-dried and ground into fine powders for the TN, TP and TOC concentration analyses. The TN and TP of sediments was determined by ultraviolet spectrophotometry. The TOC of sediments was determined using a TOC analyzer (Analytik Jena HT3100, Germany). The accuracy of TN, TP, and TOC contents in sediments was up to 0.1 mg/L level.

2.3.2. Calculate CH_4 and CO_2 emissions fluxes

The CH_4 and CO_2 emissions fluxes (F) estimated by the static chamber method are calculated as follows:

$$F = \frac{M}{V_0}\frac{P}{P_0}\frac{T_0}{T}H\frac{dc}{dt}$$

where F is the emissions fluxes (μg·m^{-2}·min^{-1}); M is the molar mass of the gas being measured (g·mol); P is the pressure at sampling (Pa), T is the temperature at sampling (K); V_0, P_0 and T_0 are the molar volume of the gas, the absolute air pressure and the temperature of the air under the standard state; H is the height above the water surface of the static box (m); dc/dt is the slope of gas concentration changing with time during sampling; it is obtained by linear regression of gas concentration at different time and corresponding time interval.

2.3.3. Measurement of CH_4 and CO_2 concentration

Gas samples from both field and microcosm collection were measured by a gas chromatograph (Aglient,7890 B).

5 mL of gas was extracted by syringe from the methane-producing potential microcosms and methane-producing pathway microcosms, and then 5 mL of N_2 was added to the anaerobic flask to maintain consistent pressure. The content of CH_4 and CO_2 was measured by a gas chromatograph (Aglient, 7890 B) with a flame ion detector (FID). The furnace temperature, FID, and ECD detector temperature were 55 °C, 200 °C, and 300 °C, respectively. 99.999% high purity nitrogen was selected as the carrier gas, and the flow rate was 2 mL/min. High purity hydrogen and air were used as the gas with flow rates of 40 mL/min and 400 mL/min, respectively. The detection limit of CH_4 was 0.2 ppm, and the detection limit of CO_2 was 4 ppm, with an error range of less than 1%. The CH_4 production capacity and efficiency of different substrates differ according to the methane-producing efficiency of different substrates (Table 1), the final CH_4 concentration was homogenized, and the specific formula was as follows:

$$C = a \times C_d/(A \times b) \quad (1)$$

$$C\% = C/(C_a + C_b + C_c) \times 100\% \quad (2)$$

where C is the concentration of CH_4 after treatment; C_d is CH_4 concentration in the system; a is the CH_4 generated coefficient; b is the number

Table 1
The chemical equation for CH_4 production and the Gibbs free energy of the reaction.

The serial number	Chemical reaction equation	Gibbs free energy δG (KJ mol^{-1} CH_4)
1	$4HCOO^- + 4H^+ = CH_4 + 3CO_2 + 2H_2O$	−130
2	$4CH_3OH = 3CH_4 + HCO_3^- + H_2O + H^+$	−223
3	$4\ R\text{–}CH_3 + 2H_2O = 4RH + 3CH_4 + CO_2$,	−113

R represents –SH, –OH, $–NH_2$, $–NHCH_3$, $–N(CH_3)_2$ or $–N(CH_3)_3^+$ (Caldwell et al., 2008; McInerney et al., 2008).

of effective groups in the substrate. C% is the proportion of each matrix pathway; C_a is CH_4 concentration via the hydrogenotrophic pathway; C_b is CH_4 concentration via the acetoclastic pathway; C_c is CH_4 concentration via the methylotrophic pathway.

2.3.4. Determination of methyl compounds

10 mL of water sample was put into a headspace flask, and the sample was enriched with a 75 μm CAR/PDMS fiber head stirred at 500 rpm for 30min at 65 °C. High purity helium (99.999%) was used as a carrier gas, and the flow rate was 1.0 mL/min. No shunt injection was carried out. The initial temperature of the column temperature box was 45 °C, and the temperature rose to 200 °C at a rate of 10 °C/min after maintaining the temperature for 3 min. The inlet temperature was 250 °C. In the full scanning mode, the ion scanning range m/z was 30–300 amu, and the scanning time was 1.5–30 min. The standards of three methyl thioether substances were purchased from Sigma-Aldrich (Milwaukee,WI, USA), and the standard solutions were diluted with MilliQ-water. The characteristic ions were M/Z of 62 for DMS, m/z 94 and 79 for DMDS, m/z 126 and 111 for DMTS.

2.3.5. Biological analysis

In order to confirm the changes of methane-producing bacteria (MPA) in the sediments from the cyanobacteria accumulation area, the open lake area, and the microcosms, the sequencing and real-time reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) technologies were used. With these technologies, the microbial communities and the cell copy numbers of MPA on days 0 (the initial stage), 12 (the intermediate stage), and 21 (the final stage) in the sediments were determined.

The sediment samples were collected and frozen at −80 °C in an ultra-low temperature freezer. The E.Z.N.A. ®Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) was used to extract the total genomic DNA from each soil sample according to the manufacturer's instructions. Nucleic acid quality and concentration were determined by 1% agarose gel electrophoresis and a NanoDrop 2000 UV spectrophotometer (Thermo Scientific, USA), respectively. The total bacteria in the sediments were quantified using 16 S rRNA gene abundance.

The MPA in the sediments were quantified using the quantitative polymerase chain reaction (qPCR) method, with primer sets targeting 1106 F (5′-TTWAGT CAG GCAACG AGC-3′) and 1378r (5′-TGT GCAAGG AGC AGG GAC-3′) in this study (Watanabe et al., 2009). The q-PCR experiments were performed on an ABI7300 qPCR instrument (Applied Biosystems, USA) using ChamQ SYBR Color qPCR Master Mix as the signal dye. Each 20 μL reaction mixture contained 2 μL of the template DNA and 16.5 μL of ChamQ SYBR Color qPCR Master Mix. Standard curves for each gene were obtained by tenfold serial dilution of standard plasmids containing the target functional gene. All operations followed the MIQE guidelines.

The universal primers MLfF (5′-GGTGGTGTMGGATTCACA-CARTAYGCWAC AGC-3′) and MLrR (5′-TTCATTGCRTAGTTWGGR-TAGTT-3′) were used to amplify bacterial 16 S rRNA genes in V3–V4 hypervariable regions for the analysis of microbial community composition and diversity (Zhu et al., 2011). MiSeq sequencing was conducted on an Illumina MiSeq sequencer (Illumina, USA) by the free online platform of Majorbio Cloud Platform (www.majorbio.com). Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UCHIME (version 7.0.1090, http://www.drive5.com/uparse/), which also identified and removed chimeric sequences.

3. Results

3.1. Physicochemical characteristics of in situ sediments and overlying water

In the overlying water, the TP concentration in the cyanobacteria accumulation area was 3.81 mg/L, much higher than the TP concentration of 1.11 mg/L in the open lake area (Table 2). Similarly, the TN concentration was 9.5 mg/L vs. 2.9 mg/L in the cyanobacteria accumulation and open lake areas, respectively. The TOC concentration in the cyanobacteria accumulation area (77 mg/L) was also much higher than the open lake area (14.7 mg/L). Chl-a displayed a great difference between areas, and reached 3124.3 μg/L in the accumulation area, while was only 124.8 μg/L in the open lake area. Owing to the cyanobacteria decay, the water in the cyanobacteria accumulation area was anaerobic with DO and Eh values of 0.06 mg/L and −304.8 mV, respectively, which were much lower than the water of the open lake area. In the sediments, the nutrient load in the cyanobacteria accumulation area was greater than that in the open lake area, specifically, the concentrations of TP, TN, and TOC in the cyanobacteria accumulation area were 2094.2, 2178.2, and 42.3 mg/kg, compared to 1312.5, 840.1, and 12 mg/kg in the open lake area, respectively.

3.2. Variations of DMS, DMDS, DMTS concentrations during the decomposition of cyanobacteria

During the decay of cyanobacteria, methyl thioethers including DMS, DMDS, and DMTS were released (Fig. 1). Results showed that the content of methyl thioethers increased with the biomass of cyanobacteria. The concentrations of DMS, DMDS, and DMTS in the 7500 g/cm^3 cyanobacteria treatment peaked at day 5 with values of 19.08, 1.14, and 1.02 μg/L, respectively. While in the 25,000 g/cm^3 cyanobacteria treatment, DMS peaked at day 2 with a concentration of 96.35 μg/L, DMDS peaked at day 3 with a concentration of 3.22 μg/L, and DMTS peaked last at day 8 with a concentration of 13.61 μg/L.

3.3. Carbon output from the non- and cyanobacteria accumulation areas

The potential of CH_4 production from the sediment of the cyanobacteria accumulation area was significantly higher than that in the open lake, and the CH_4 content increased rapidly from the 13th to the 31st day of culture (Fig. 2). The potential of CH_4 production in the sediment of the cyanobacteria accumulation area increased from 170.36 μmol/L to 1199.26 μmol/L, and then remained stable, while it increased from 107.72 μmol/L to 205.76 μmol/L, and then remained stable in the open lake. Similarly, the CO_2 emissions from the sediment in the cyanobacteria accumulation area were significantly higher than those in the open lake. In microcosm systems, the final CO_2 content in the sediment of cyanobacteria accumulation area was up to 2072.8 μmol/L, while it was 714.62 μmol/L in the open lake. The carbon output from the cyanobacteria accumulation area was greater than that from the non-cyanobacteria accumulation area (Fig. 3). Specifically, in the field, the CO_2 emission flux of the cyanobacteria accumulation area was 6920.63 μg/m^2·min, while it was −726.29 μg/m^2·min^{-1} in the open lake. The CH_4 emission flux was much lower than the CO_2 flux with the average values of 3.44 μg/m^2·min and 867.01 μg/m^2·min in the non- and cyanobacteria accumulation areas, respectively.

3.4. Substrate source for CH_4 production in eutrophic lakes

Under three different substrate conditions, the CH_4 content increased gradually and remained stable after 20 days of culture (Fig. 4). Among the three substrate treatments, the treatment of $N(CH_3)_3$ had the highest final yield of CH_4, and the cumulative concentration of CH_4 in the system reached 1860.11 μmol/L. This was followed by the substrate treatment of CH_3COONa and that of HCOONa with their cumulative concentrations being 1347.85 μmol/L and 257.13 μmol/L, respectively. Based on the calculation of CH_4 production efficiency, there were dynamic changes in CH_4 production depending upon the substrate type. Among the initial CH_4 production pathways, the hydrogenotrophic pathway was dominant, accounting for 88.42% of CH_4 production, followed by the acetoclastic pathway (11.31%) and then the methylotrophic pathway (only 0.27%). The proportions of the three pathways all

Table 2
Initial physicochemical indices of the sediments and water in the open lake area and cyanobacteria accumulation area.

	Sample	TP	TN	TOC	Chl-a	DO	Eh
Open lake area	water	1.1 mg/L	2.9 mg/L	14.7 mg/L	124.8 μg/L	5.29 mg/L	172.8 mV
	sediment	1312.5 mg/kg	840.1 mg/kg	12.0 mg/kg	–	–	–
Cyanobacteria accumulation area	water	3.8 mg/L	9.5 mg/L	77.0 mg/L	3124.3 μg/L	0.06 mg/L	−304.8 mV
	sediment	2094.2 mg/kg	2178.3 mg/kg	42.3 mg/kg	–	–	–

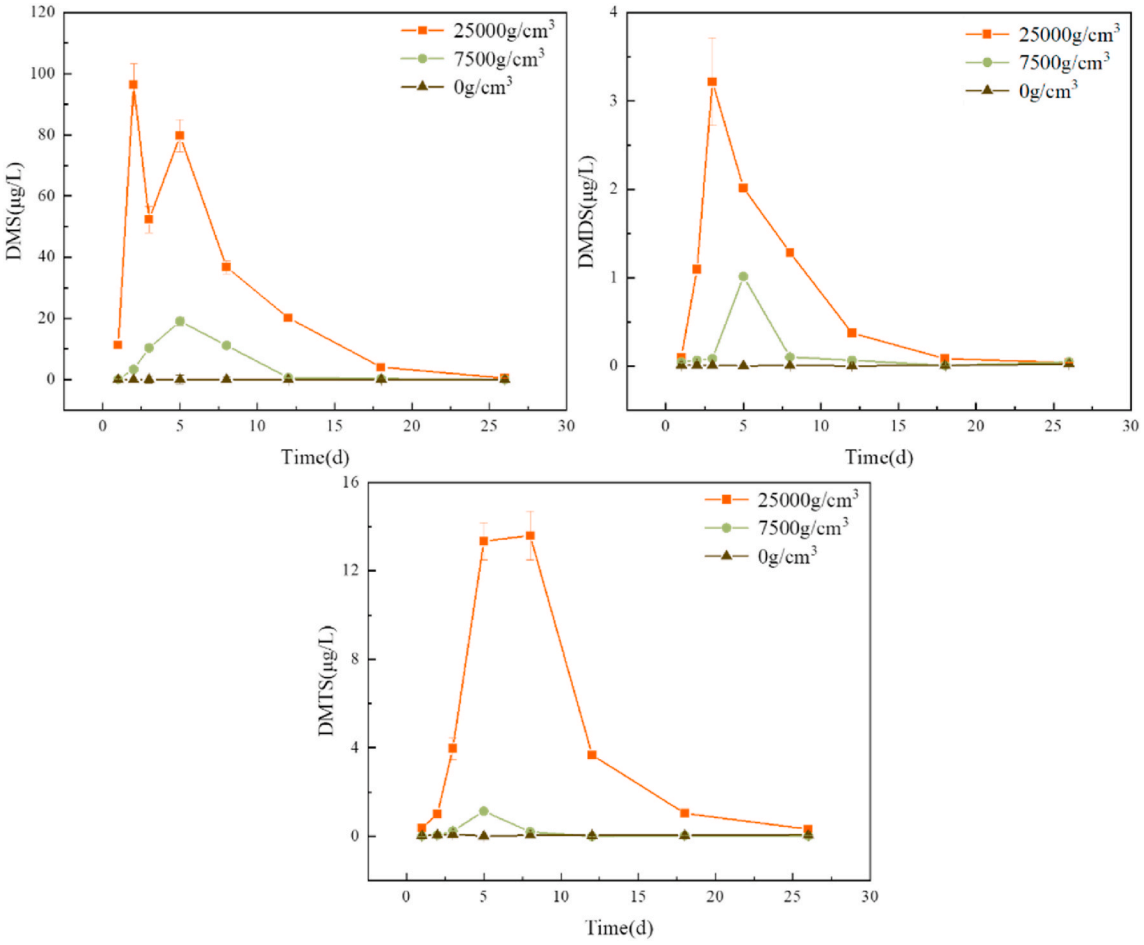

Fig. 1. Dynamics of DMS, DMDS and DMTS concentrations during the decomposition processes of cyanobacteria detritus from Lake Taihu incubated in the microcosms with different biomass under anaerobic conditions.

changed significantly and reached a relatively stable level from the 6th day until the end of the culture. Therefore, the final proportions of the hydrogenotrophic pathway, acetoclastic pathway and methylotrophic pathway were 32.58%, 41.68%, and 25.74%, respectively.

3.5. Microbial communities and quantification of MPA in sediments

As showed in Fig. 5 and Fig. 6, there were significant differences between the abundance of cyanobacteria and the relative abundance of each species in the cyanobacteria accumulation area and the open lake area. In both areas, Methanosarcinales, Methanomassiliicoccales, Methanocellales, Methanobacteriales, and Methanomicrobiales were all present. Methanobacteriales accounted for the highest proportion of the microbial community in the sediments from the cyanobacteria accumulation area and the open lake area, however, the proportion of Methanobacteriales differed between the two areas (22.94% vs. 35.08%). In the cyanobacteria accumulation area, the second dominant methanogen was Methanomicrobiales, which accounted for 10.94%. Methanomassiliicoccales and Methanosarcinales accounted for 6.52% and 6.23%, and Methanocellales were the least abundant in the cyanobacteria accumulation area, with a proportion of 1.63%. In the sediments from the open lake area, Methanomassiliicoccales accounted for 27.55% of the microbial community, Methanomicrobiales were 11.43%, and Methanomassiliicoccales and Methanocellales were relatively low in abundance with only 0.44% and 0.01%, respectively.

In the initial sediments of the microcosm, Methanobacteriales were the dominant species making up 26.24% of the microbial community, followed by the Methanomicrobiales with 10.37% abundance, and then Methanomassiliicoccales with 9.34%, Methanosarcinales with 7.67%, and Methanocellales with 1.18% abundance. In the control, there was no significant change in the microbial composition between days 15 and 21. Methanobacteriales were the dominant species on both days 15 and 21, however, on day 21, the relative abundance of Methanosarcinales (13.69%) was slightly higher than that of Methanomicrobiales (12.38%). In the treatment of HCOONa, there was no significant change in the composition of bacteria, and the dominant species on both days 15 and 21 were the Methanobacteriales, accounting for 40.42% and 30.10% abundance, respectively. The relative abundance of

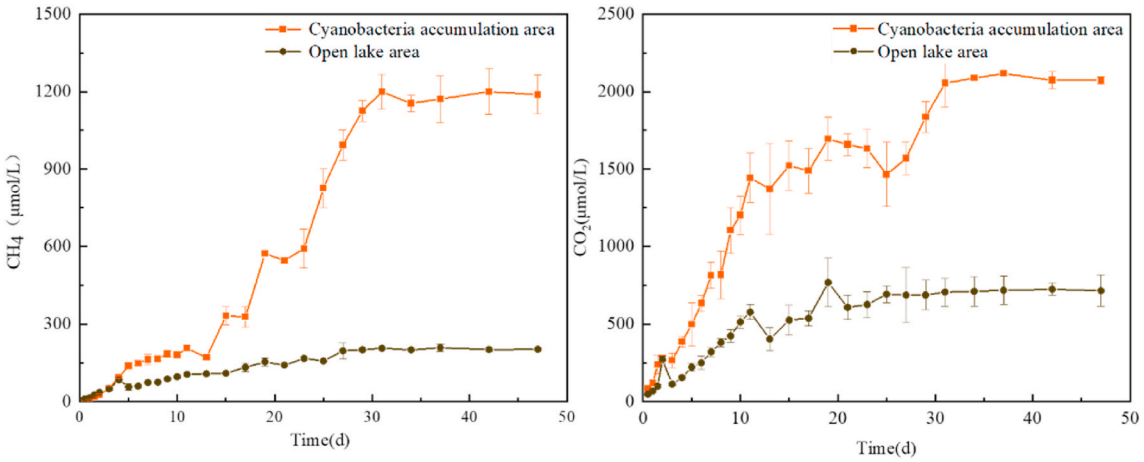

Fig. 2. CH_4 and CO_2 emission potential of sediments from cyanobacteria accumulation area and open lake area in Lake Taihu incubated in the microcosms under anaerobic conditions.

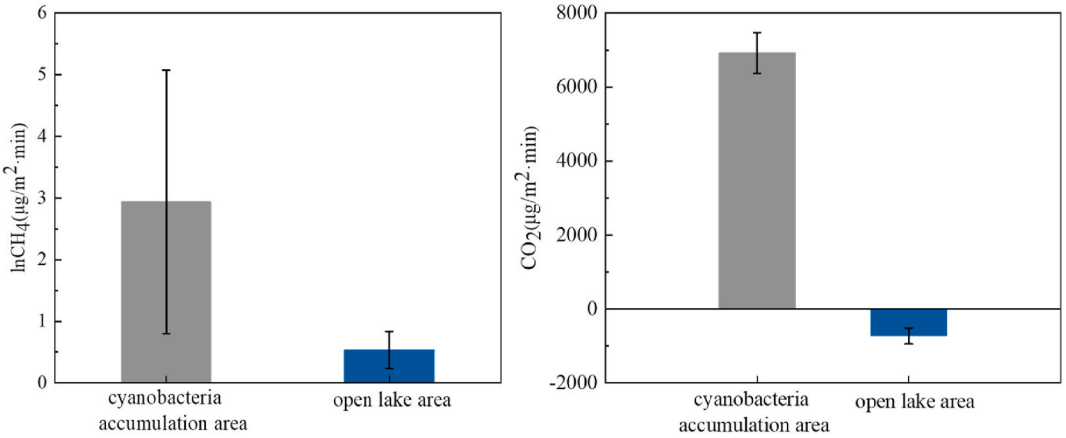

Fig. 3. CH_4 and CO_2 release fluxes across water-air interface of cyanobacteria accumulation area and open lake area in Lake Taihu collected via floating static chambers.

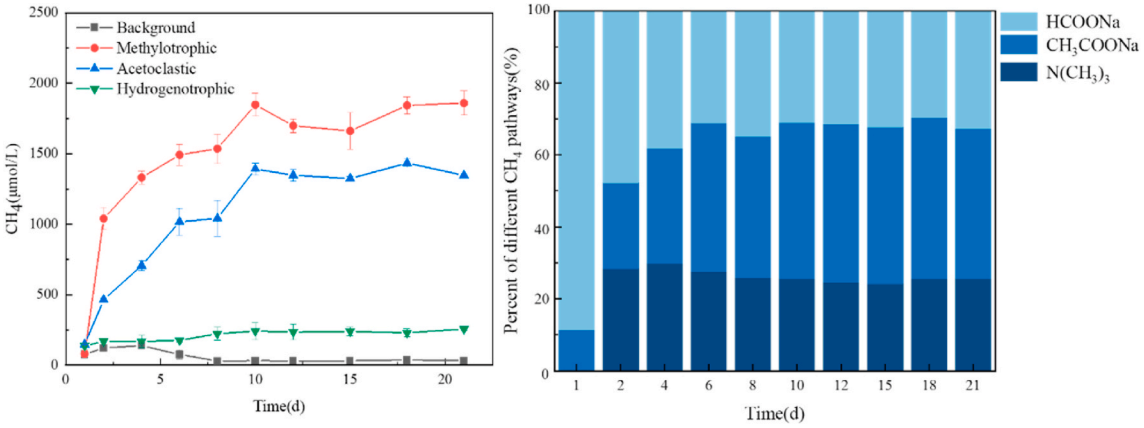

Fig. 4. Dynamics (left) and the relative proportion (right) of CH_4 concentrations produced from incubated sediments of Lake Taihu in the microcosms under anaerobic conditions via adding CH_3COONa, HCOONa, and $N(CH_3)_3$ substrates for determining acetolactic, hydrogenotrophic, and methylotrophic methane production pathways, respectively.

Methanomicrobiales increased significantly from 10.37% at the beginning to 15.96% on day 15, and the proportion was 21.51% at the end of culture. In the treatment of CH_3COONa, the methanogens' abundance varied greatly. Particularly, Methanobacteriales decreased significantly from the initial 26.24% abundance to 17.67% on day 15, and only 6.64% abundance at the end of culture. Methanosarcinales increased significantly from an initial abundance of 7.67%–13.14% on day 15, and accounted for 36.02% of the microbial community at the end of culture. There was no significant change in the proportions of the other methanogens. In the treatment of $N(CH_3)_3$, Methanobacteriales were the

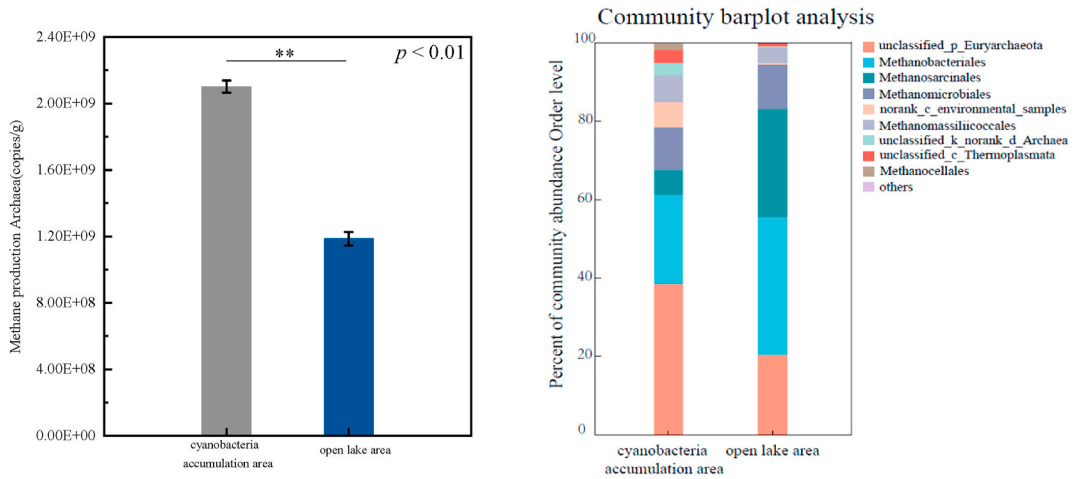

Fig. 5. The copies of methanogenic archaea (left) and the community structure (right) of MPA in the surface sediments of cyanobacteria accumulation area and open lake area in Lake Taihu.

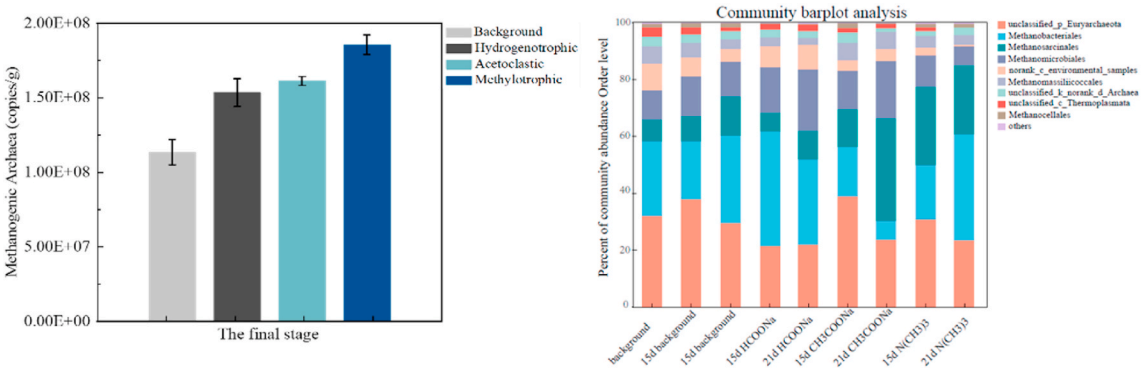

Fig. 6. The copies of methanogenic archaea (left) and the community structure (right) of MPA in the incubated sediments of Lake Taihu treated with different substrates in the microcosms under anaerobic conditions.

dominant strain, and increased considerably, with the proportion going from only 7.67% at the initial stage, to 27.43% on day 15 and 24.31% at the end of culture.

As shown in Figs. 5 and 6, based on the results of RT-qPCR, the MPA number exhibited obviously different characteristics in all samples. The MPA number in the cyanobacteria accumulation area was 77.33% higher than that in the open lake. At the final stage in indoor simulation, there are differences among different substrates. The MPA number was highest in $N(CH_3)_3$ substrate type, 17.3% higher than HCOONa substrate type and 15.05% higher than CH_3COONa substrate type.

4. Discussion

Freshwater systems make up only a small part of the land surface, however, they are one of the largest sources of carbon emissions in the atmosphere, among which, the release of CH_4 into the atmosphere is up to 117–212 Tg/y (Saunois et al., 2020; Rosentreter et al., 2021). However, carbon emissions exhibit significant differences between lakes and are influenced by temperature, nutrient status, and the abundance of MPA, etc (Bastviken et al., 2004; Zhou et al., 2020). The hypereutrophic Lake Taihu is reported to have a CH_4 flux of up to 2106.3 mmol m^{-2} yr^{-1}, which is higher than the average CH_4 flux of 531.5 mmol m^{-2} yr^{-1} in other Chinese lakes (Yang et al., 2011). It has been documented that the mean CH_4 emission fluxes from mesotrophic, eutrophic, middle-eutrophic, and hyper-eutrophic lakes are 1.67, 73.3, 200, and 2173.3 μg m^{-2}·min^{-1}, respectively (Zhou et al., 2020). In this study, the CH_4 emission flux in a severe cyanobacteria accumulation area of Lake Taihu reached a flux of 867.01 μg m^{-2}·min^{-1} (Fig. 3), distinctly higher than other lakes. Nowadays, the frequent outbreak of cyanobacterial blooms has become a major management problem facing freshwater lakes (Zhang et al., 2020; Song et al., 2021). In most eutrophic lakes, cyanobacteria blooms are commonly driven by wave and wind, resulting in the accumulation in near-shore areas or being trapped in emergent macrophytes (Xing et al., 2011; Yan et al., 2017; Ma et al., 2020). These accumulated cyanobacteria blooms easily die off with nutrient or light competition, resulting in the formation of a serious siltation layer of cyanobacteria residue on the surface sediment (Huang et al., 2018a,b; Qi et al., 2020). The decomposition of cyanobacteria promotes the accumulation of organic matter and nutrients within the sediment and induces changes in the microbial community structure, often with an increase in the MPA abundance (Fig. 4). Studies have shown that more CO_2 flux represents more active biological processes (Ma et al., 2020). In this study, the more CO_2 flux in the cyanobacteria accumulation area demonstrated more active biological processes (Fig. 3), while it was negative in the open lake caused by the photosynthesis in small amounts of cyanobacteria (Cabrerizo et al., 2020). The final result from such cyanobacteria blooms is an increase in CH_4 emissions (Yan et al., 2017; Tong et al., 2021). Therefore, the CH_4 emissions in the cyanobacteria accumulation area were significantly higher than that in the open lake center where there were less cyanobacteria.

It has become a consensus that the outbreak of cyanobacteria blooms promotes CH_4 and CO_2 emissions, and subsequently creates a positive feedback, which forms a vicious loop (Yan et al., 2017; Mullin et al., 2020; Tong et al., 2021). Many studies have paid a lot of attention to CH_4

collection methods and emission fluxes in eutrophic lakes (Bastviken et al., 2004; Yan et al., 2017), however, the CH_4 producing pathways are still far from clear, especially in areas that exhibit severe cyanobacteria accumulation. It is generally accepted that the hydrogenotrophic and aceticlastic reactions are the main pathways of methanogenic production in freshwater lakes due to the low content of methyl compounds (Preheim et al., 2016). Recently, in some extreme lacustrine environments, i.e., polar and plateau salt, methylotrophic CH_4 producing pathways have been found to be important sources of CH_4 production (Deng et al., 2017; Liu et al., 2017). In addition, it has been reported that cyanobacteria can mediate CH_4 production in oxic conditions when methionine, a precursor of several methylated molecules as DMS, is added (Morana et al., 2020). In this study, we found evidence for severe cyanobacteria accumulation inducing methylotrophic CH_4 producing pathways in eutrophic lakes. During the decay of cyanobacteria, a large amount of methyl thioethers, including DMS, DMDS, and DMTS, were released, and their concentrations increased with the biomass of cyanobacteria (Fig. 1). At the initial anaerobic decomposition process of cyanobacteria, proteins, carbohydrates, and other complex organic matters in algal cells are converted into amino acids and polypeptides after hydrolysis and anaerobic fermentation. After further decomposition via microorganisms, the small molecules H_2, CO_2, DMS, DMTS, and Methyl mercaptan, etc., are released (Zinder et al., 1997; Huang et al., 2018a,b). During these biological processes, dimethyl sulfonio propionate is present in the somatic cells of the cyanobacteria and enters the water body after cell death and rupture, where it finally forms methylated sulfide substances through microbial action and methylation, which is consistent with the results of previous studies (Liu et al., 2017; Huang et al., 2018a,b). When methyl compounds are abundant, it is possible to induce CH_4 production through the methylotrophic pathway (Figs. 4 and 6). In addition, widespread evidence has revealed that the production and emission of CH_4 are associated with microbial processes (Bastviken et al., 2008; Borrel et al., 2011). Microbiological data showed that both the cyanobacteria accumulation area and the open lake area had Methanosarcinales and Methanomassiliicoccales which can produce CH_4 using the methylotrophic pathway (Fig. 5). The amount of MPA in the cyanobacteria accumulation area was also found to be 77.33% higher than that in the open lake area (Fig. 5). Methanosarcinales and Methanomassiliicoccales can produce CH_4 via the methylotrophic pathway, by using methyl compounds, methylamine compounds, and methyl sulfur compounds (Franzmann et al., 1992; Liu et al., 2008). However, Methanomassiliicoccales possess unique metabolic properties, since they lack a complete pathway to reduce CO_2 to methyl coenzyme M, and need additional hydrogen to grow (Franzmann et al., 1992). The release of complex and unstable substances during the decomposition process of cyanobacteria results in changes in the diversity and structure of the microbial community. Field data showed that Methanosarcinales, Methanomassiliicoccales, Methanocellales, Methanobacteriales, and Methanomicrobiales were present in sediments, with the proportion of each methanogen differing (Fig. 5). A microcosm simulation showed that there was sufficient substrate, and CH_4 could be produced by three pathways, as evidenced by the constant proportion of the three pathways in the later period of the culture (Fig. 4). In parallel, the microbial community structure in the sediments showed obvious changes throughout the culture period. The culture group with $N(CH_3)_3$ as a substrate had Methanobacteriales as the dominant species, although the abundance of Methanosarcinales increased significantly throughout the culture period (Fig. 6). This may be due to sufficient $N(CH_3)_3$ causing an increase in the abundance of Methanosarcinales (Borrel et al., 2011). In addition, there are many other reasons for differences in the prevalence of different CH_4 production pathways, including nutrients, DO, etc (Preheim et al., 2016; Zhao et al., 2019). Field data showed that there were significant differences in CH_4 emissions and the MPA community structure and abundance between different habitats (Table 2; Fig.5; Fig.6). Cyanobacteria accumulation and decomposition have been shown to affect these indicators (Table 2) and can impact the structure of the microbial community (Fig. 5).

To clarify the relationship between severe cyanobacteria accumulation and its ability to potentially induce methylotrophic CH_4-producing pathways in eutrophic lakes, a conceptual diagram was put forward. In this diagram, the obvious differences between the cyanobacteria accumulation area and the open lake area can be seen (Fig. 7). A distinct decrease in water DO, as well as a considerable increase in TN, TP, and TOC, indicated aquatic hypoxia and an anaerobic, reductive microenvironment (Chuai et al., 2011; Chen et al., 2014). Cyanobacteria decomposition produced abundant $N(CH_3)_3$ (Fig. 1), formed anaerobic microenvironments (Table 2), and significantly increased the relative abundance of Methanosarcinales (Figs. 5 and 6). These changes finally promoted the production and emission of CH_4 in lake sediments. The laboratory experiment highlighted that when $N(CH_3)_3$ was sufficient, the final proportion of CH_4 produced reached 32.58% (Fig. 3). At the same time, an increase in the SO_4^{2-} concentration in the lake may also be one of the reasons for the existence of the methylotrophic pathways (Yu et al., 2013). Nutrients released during the decay and decomposition of cyanobacteria promote the growth of anaerobic fermentation bacteria (Zhao et al., 2019), including increasing the abundance of MPA (Fig. 5). As a result, the CH_4 emission capacity of the cyanobacteria accumulation area was significantly greater than that of the open lake area (Fig. 3).

It has been proven in this experiment that severe accumulation of cyanobacteria may induce methylotrophic CH_4 producing pathways. According to the experimental results of this study, under the condition of sufficient methyl compounds, the CH_4 emission flux of the methylotrophic pathway in the cyanobacteria aggregation area of Taihu Lake reached 2254.73 $\mu g\ m^{-2}\cdot min^{-1}$. This is of great significance to the knowledge of CH_4 pathways within eutrophic lakes. In future assessments of the CH_4 production capacity of eutrophic lakes, we need to take into account the presence of CH_4 production by methylotrophic pathways induced by severe cyanobacteria blooms, and its overall influence on CH_4 emission.

5. Conclusions

The outbreak of cyanobacteria blooms significantly increased the contents of C, N, and P in overlying water and sediments, and promoted the emission of CH_4 and CO_2 in sediments. The potential of CH_4 and CO_2 generation in sediments in the area with cyanobacteria accumulation was much higher than that in the open lake. In this study, severe accumulation of cyanobacteria promoted the increase of MPA abundance and a change in the microbial community structure. The number of MPA in the cyanobacteria accumulation area was 77.33% higher than that in the open lake area, and Methanobacteriales were the dominant species. During the decomposition of cyanobacteria, methyl thioethers, such as DMS, DMDS, and DMTS, were released, with their concentration increasing with an increase in cyanobacteria biomass. The experimental data showed that when methyl compounds were abundant, methylotrophic methane-producing pathways were induced. We, therefore, have reason to believe that severe accumulation of cyanobacteria blooms may induce methylotrophic methane-producing pathways in eutrophic lakes, which has important significance for the evaluation of the CH_4 production capacity of eutrophic lakes.

Author statement

Xu Xiaoguang: designed and led the study. Zhou Chuanqiao, Peng Yu, Yu Miaotong, Deng Yang, Chen Li, Zhang Lanqing, Zhang Siyuan, and Yan Yan: performed the investigation and analyzed the samples. Zhou Chuanqiao, Peng Yu: wrote the original draft with major edits and inputs from Xu Xiaoguang and Wang Guoxiang.

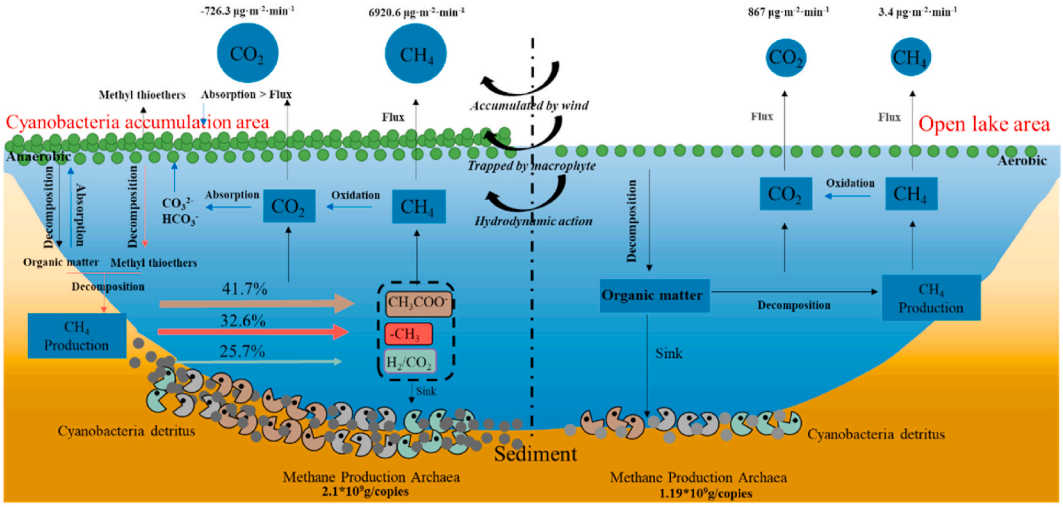

Fig. 7. A conceptual diagram of CH_4 production potential, pathway and emission flux in severe cyanobacteria accumulation area and the open lake. In eutrophic lakes, cyanobacteria blooms are easily trapped by emergent macrophytes and accumulate in bays or near-shore areas driven by the wind or hydrodynamic action. The severe cyanobacteria accumulation produces abundant methyl thioether substances, including DMS, DMDS, and DMTS, forms extremely anaerobic conditions, and potentially induces methylotrophic methane producing pathway in eutrophic lakes coupled with the increasing abundance of MPA. These biochemical processes also increase the CH_4 emission fluxes in severe cyanobacteria accumulation areas.

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.

Acknowledgements

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