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Nitrogen recovery from wastewater to produce microbial protein using methane oxidizing bacteria

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Abstract: Nitrogen removal is one of the central objectives in contemporary wastewater treatment. However, traditional nitrogen removal treatment processes have high energy demand and involves microbial conversion of reactive nitrogen (ammonium, nitrite, and nitrate) to inert nitrogen gas [1]. Recently, nitrogen recovery from wastewater has attracted attention, as it is benefit for both water pollution control and nitrogen cycle optimization. About 2% of the total world energy consumption is used to produce reactive nitrogen via the Haber Bosch process (industrial production of N fertilizers) [2]. Substituting just 5% of N fertilizer production by N recovery from wastewater would save more than 50 terawatt-hours of energy, about 1.5% of China's annual electricity consumption [1]. So far, common nitrogen recovery technologies include struvite precipitation, adsorption and electrodialysis, none of which are economically competitive. Bacteria and algae can synthesize valuable feed-grade microbial protein by highly efficient nitrogen assimilation. Consequently, nitrogen removal from wastewater towards microbial protein production can be considered. In particular, methane oxidizing bacteria (MOB) utilize methane, which is cheap and can be easily generated from anaerobic digestion, as carbon and energy source, whilst residual ammonia is assimilated and converted to protein. Therefore, nitrogen recovery from wastewater to produce MOB protein has both technical and economic advantages. Through MOB growth, the microbial protein production could cost much less energy than protein production through the conventional route (such as feed beef cattle) (ca. 360 MJ/kg N-protein vs 4000 MJ/kg N-protein [2]). MOB protein has been successfully used as feed ingredient for monogastric animals (such as pigs, broiler chickens and Atlantic salmon) [3]. However, efficient and safe oxygen and methane supply remains a bottleneck for high-rate MOB cultivation. In this study, an innovative bubble-less membrane-based reactor (Fig. 1) employing hollow-fiber membranes was developed to produce MOB protein using ammonium from synthetic wastewater. Methane and air are supplied separately to the reactor using highly-efficient hydrophobic hollow-fiber membrane modules (Mitsubishi Rayon Co., Ltd., Tokyo, Japan). In this way methane and oxygen are only in contact solubilized in the liquid phase, thereby avoiding the formation of explosive atmosphere inside the reactor. An MOB enrichment reported by Van der Ha et al. [4] was inoculated into the reactor. Batch cultivation was carried out to activate biomass in the startup phase (1 week) followed by continuous flow operation. Synthetic wastewater containing 28-140 mgNH_4^+-N/L was used as influent, with trace

nutrients and phosphate buffer [4]. Hydraulic and solid retention times are set at 2 days whilst temperature is kept at 20-22 ºC. The performance of startup phase is shown in Fig.2. The results showed that highly efficient and safe methane and oxygen supply achieved in this reactor, with oxygen transfer coefficient (K_La) higher than 2 h^{-1} in the case of only 1 bundle (of 96 fibers) installed. MOB achieved a growth rate higher than 1.5 d^{-1}. The methane-based yield was 0.78 mgVSS/mgCH_4, whilst the nitrogen-base yield was 6.2 mg VSS/mgNH_4^+-N. CH_4 is the limiting factor in the startup phase. The growth rate of MOB will be increased by increasing the methane supply. The reactor will be operated under different NH_4^+-N/O_2/CH_4 ratios to define the optimal operational conditions to enhance biomass productivity and protein accumulation. At the end of each stage, total protein and amino acid profile will be tracked to evaluate protein productivity and quality. The microbial community dynamics will be analyzed via Illumina sequencing and targeting *pmo*A functional genes. This study provides proof-of-concept of a novel technology for nitrogen recovery from wastewater into methanotrophic microbial protein.

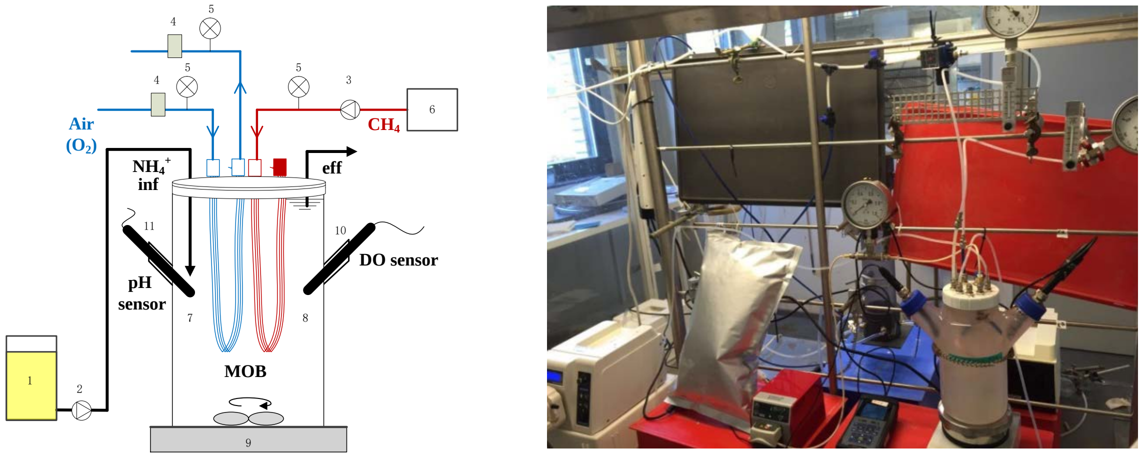

Fig.1. The schematic diagram (left side) and picture (right side) of the bubbleless membrane-based reactor (2.7 L) for producing MOB protein. (1, influent tank; 2, influent pump; 3, methane supply pump; 4, air flow meter; 5, manometer; 6, methane storage bag; 7, hollow fiber for air supply; 8, hollow fiber for methane supply; 9, magnetic stirring; 10, DO sensor; 11, pH sensor.)

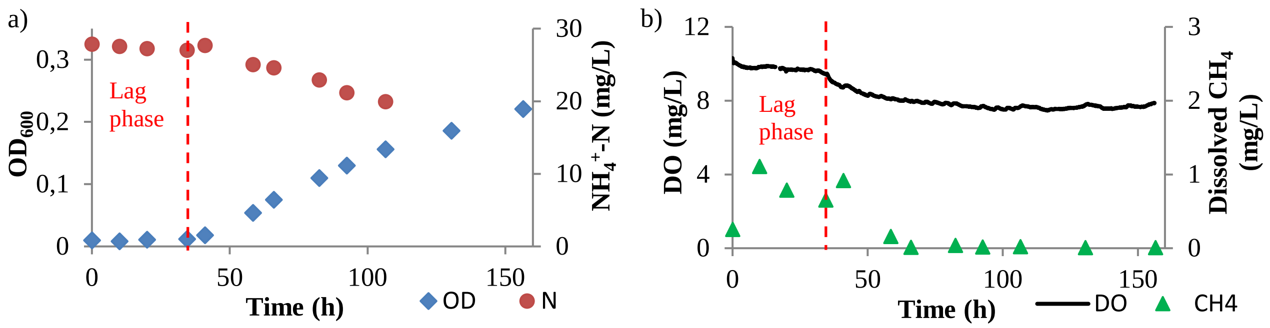

Fig.2. The performance of startup phase (batch operation) in the bubbleless membrane-based reactor. a) Optical density at 600 nm (OD_{600}) and the concentration of NH_4^+-N in the reactor; b) Dissolved oxygen (DO) and dissolved CH_4.

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