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Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

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Keywords: Single Cell Protein, Methane Oxidizing Bacteria, Bubble-free Gas Supply, Nutrient Recovery.
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Global population increase, climate change and industrialization are leading to alarming depletion rates of many resources worldwide. Consequently, there is an increasing interest on recovering resources from waste streams, which have been traditionally not utilized or negatively valued (Foley et al., 2011). Conventional strategies for valorisation of bio-waste focus mainly on the production of biogas and recovery of nutrients as, e.g., compost or slurries that can be used as fertilizers. However, fertilizer application on land is not efficient because it leads large nutrient losses (about 50% of the total nitrogen input) as greenhouse gas emissions or due to run-off, thereby contaminating ground water. Most of the produced crops are used as protein source for livestock consumed by humans. Therefore, recovery of proteins from waste, rather than fertilizers, is desirable (Matassa et al., 2015).

Single-cell protein (SCP) consisting of microbial biomass can generate nutritive proteins, with quality equal or better than conventional protein sources (e.g. soy or fishmeal) at a lower cost. Some attempts have been made to produce SCP from synthetic or fossil fuel based resources (referred to as first generation processes). As example, methane oxidizing bacteria (MOB) are used at large scale for SCP production using natural gas and synthetic nitrogen sources. MOB can be grown in effluents from anaerobic digestion, enabling nutrient and carbon recovery into valuable feed ingredients. However, there are still limitations related with gas supply, which is usually inefficient and creates explosive mixtures of methane and oxygen (Petersen et al., 2017). In this study we developed two different approaches for safe gas supply to methanotrophic cultures for SCP production.

In the first case, we tested the use of green microalgae (*Chlorella sorokiniana*) to provide oxygen to MOB (*Methylococcus capsulatus*) when cultivated in industrial wastewater and fed with synthetic biogas (Fig. 1a). The reactor was run as batch and in triplicates. Results demonstrated that both algae and bacteria could remove or assimilate most of the organic carbon present in the wastewater. However, their growth stopped before nutrients and substrates in the gas phase (i.e., methane and oxygen for methanotrophs and carbon dioxide for algae) were depleted. Likely, algal growth was light limited and stopped after organic carbon was consumed, whilst growth of methanotrophic bacteria could be limited by trace elements (e.g., copper). Nevertheless, the amino acid profile of both the monocultures and the algal-bacteria consortium was suitable for substitution of conventional protein sources (Fig. 2A; Schøyen et al., 2007).

For the second case, an innovative bubble-free membrane-based reactor (Fig. 1b), employing hollow-fibre membranes, was developed to produce MOB protein using ammonium from synthetic wastewater. The system was started-up as a batch reactor and then operated as a continuous flow system. Methane and air were supplied separately to the reactor using highly-efficient hydrophobic hollow-fibre membrane modules (Mitsubishi Rayon Co., Ltd., Tokyo, Japan). In this way methane and oxygen were only in contact solubilized in the liquid phase, thereby avoiding the formation of explosive atmosphere inside the reactor. When oxygen was supplied from air, exhausted gas phase built up on the top of the reactor, which contained mainly nitrogen and some traces of methane. Furthermore, the oxygen level in the liquid was relatively low (approx. 0.1 mg L^{-1}), which led to biofilm growth in the membranes. Pure oxygen supply minimized the creation of a gas phase in the reactor. Furthermore, increasing the oxygen level above 0.5 mg L^{-1} supported microbial growth in suspension and fouling was minimized. The mixed culture grown in the system was characterized by using Illumina sequencing, which showed that *Methylobacter* was the main MOB growing in the system, followed by *Methylomonas*. Interestingly, both could grow in the system even when methane supply was stop. MOB probably oxidized ammonia to nitrite in the absence of methane. The mixed culture stored good quality protein, which could serve as an alternative protein source for, e.g., fish (Fig. 2B; Yamamoto et al., 2005).

Both approaches showed promising results, as the biomass produced can be included in diets for different animals consumed by humans, therefore helping to ensure food supply. Further studies should focus on

optimizing the activity balance between algae and MOB for the first approach, so oxygen does not accumulate in the system. Furthermore, both systems have to be tested with real biogas in the future.

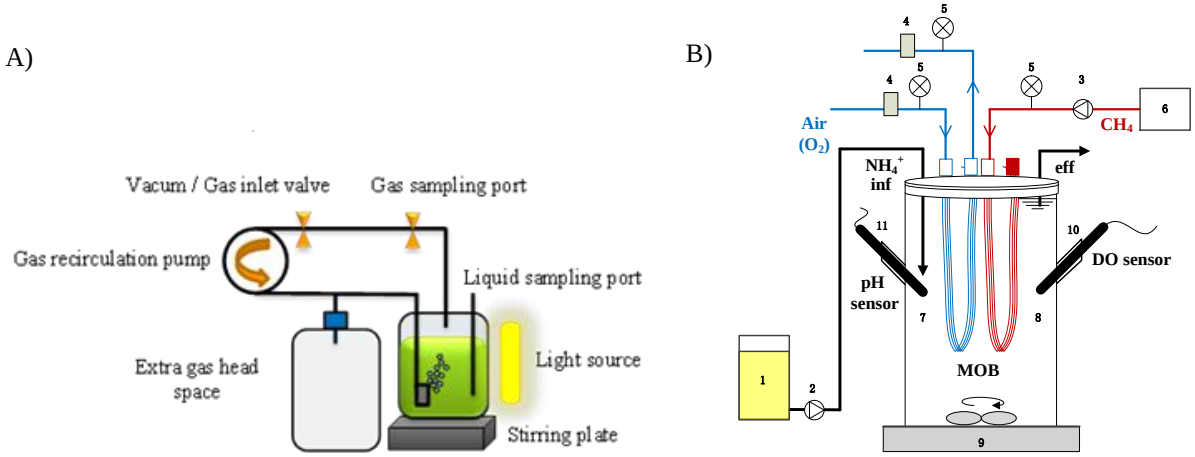

Figure 1. A) Schematic representation of the photobioreactor used for cultivation of the bacteria-algae consortium; B) bubble-free membrane-based reactor used for cultivation of the mixed MOB culture.

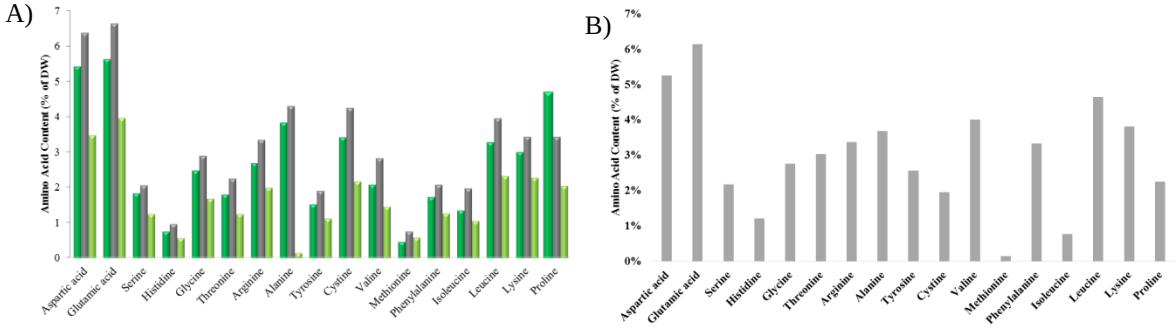

Figure 2. A) Amino acid profile of *Chlorella sorokiniana* (dark green), *Methylococcus capsulatus* (grey) and algal-bacterial consortium (light green) cultivated in industrial wastewater; B) amino acid profile of the mixed culture grown in the bubble-free bioreactor.

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