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# Single cell protein production using biogas and effluent produced from anaerobic digestion of urban bio-waste

Panagiotis Tsapekos<sup>a</sup>, Benyamin Khoshnevisan<sup>a,b</sup>, Borja Valverde-Pérez<sup>a</sup>, Yifeng Zhang<sup>a</sup>, and Irimi Angelidaki<sup>a</sup>

<sup>a</sup>Department of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby, DK-2800, Denmark

<sup>b</sup>Department of Mechanical Engineering of Agricultural Machinery, Faculty of Agricultural Engineering and Technology, College of Agriculture and Natural Resources, University of Tehran

(E-mail: [ptsa@env.dtu.dk](mailto:ptsa@env.dtu.dk); [b\\_khoshnevisan@ut.ac.ir](mailto:b_khoshnevisan@ut.ac.ir); [bvape@env.dtu.dk](mailto:bvape@env.dtu.dk); [yifz@env.dtu.dk](mailto:yifz@env.dtu.dk); [iria@env.dtu.dk](mailto:iria@env.dtu.dk))

## Abstract

In this research, a novel concept is suggested to upgrade the underutilized urban bio-waste into single cell protein (SCP) using raw or biologically upgraded biogas as well as nutrients extracted from anaerobic digestate. Specifically, more than 0.78 gTSS/gCH<sub>4</sub> were produced using biologically upgraded biogas. In addition, more than 92% nitrogen assimilation was achieved by methanotrophs when an electro dialysis cell was used. Results demonstrated that the production of SCP using nutrients and biogas streams from anaerobic digestion of urban bio-waste can stand as alternative to the usage of synthetic nitrogen sources and pure gases.

## Keywords

Urban bio-waste; Single Cell Protein; Circular bio-economy

Session – Resource recovery

## INTRODUCTION

A significant portion of agricultural land is devoted to the cultivation of proteinaceous crops as feed for livestock production and aquaculture. Nowadays, there is an imperative demand to find efficient and innovative ways to produce protein rich food sources to meet the world's demands. On this topic, microbial protein production known also as single cell protein (SCP) is considered as a potential solution to address the aforementioned challenges (Matassa et al., 2016).

Regarding bacteria-based SCP, methanotrophs using methane as their only source of carbon and energy are suitable microbes for animal feed production. To achieve a low cost SCP production able to compete with traditional agriculture, inexpensive feedstocks appear as the only choice. On this topic, abundant urban bio-waste have not yet been tested for SCP production using methanotrophs.

The aim of this study was to demonstrate the proof-of-concept for SCP from urban bio-waste. Different strategies were developed to define the microbial protein production, including cultivation of methanotrophs in: a) the liquid fraction of centrifuged and filtered digestate, b) extracted nitrogen from digestate using an electro dialysis cell. In addition, the effect of methane source was investigated.

## MATERIALS AND METHODS

### Mixed methanotrophic culture

A mixed methanotrophic consortium was grown for CH<sub>4</sub> and NH<sub>4</sub> assimilation using diluted ammonium mineral salt (dAMS) medium. All batch fermentations were inoculated with 3% (v/v) of methanotrophic inoculum and kept in a shaker incubator (150 rpm) at 30 °C. The gas in the headspace was kept at a 2:1 oxygen to methane ratio.

### Nutrients recovery and methane sources

Urban bio-waste was anaerobically digested and the effluent was used to grow SCP. Different nitrogen recovery methods were applied:

1) *Centrifuge filter system*: The effluent was centrifuged and the supernatant was filtered and pasteurized. The liquid was diluted to reach the predetermined ammonium concentration.

2) *EDC-Recovery*: A two chamber electro dialysis cell (EDC) was used and the content of trace elements was manually adjusted to comply with dAMS composition.

Two different types of methane sources were tested: 1) Biogas produced from urban bio-waste (~60% CH<sub>4</sub>) and 2) Biologically upgraded biogas (~95% CH<sub>4</sub>).

## RESULTS AND DISCUSSION

### Effect of nitrogen source on SCP production

To begin with the "*Centrifuge filter system*", an OD of 0.451 was obtained (Fig. 1). Based on the results, the ammonium assimilation was ~62%. Regarding the gas consumption, biomass yield in terms of methane ( $Y_{CH_4}$ ) was  $0.70 \pm 0.04$  gTSS/gCH<sub>4</sub>. The tests using the centrifuged-filtered digestate produced more bacterial biomass than the control using dAMS. In the experimental set where, the nitrogen was recovered using the electrochemical reactor (*EDC-recovery*), the ammonium recovery was markedly high (~92%) and subsequently, the  $Y_{CH_4}$  was  $0.66 \pm 0.03$  gTSS/gCH<sub>4</sub>. Despite the slightly lower biomass yield in terms of methane that was achieved in *EDC-recovery* compared to *Centrifuge filter system*, more bacterial biomass was produced in the second approach (OD=0.608). After *EDC-recovery* the fermentation medium was supplemented with extra nutrients and trace elements according to dAMS composition. Thus, the composition was rich in nutrients favoring the bacterial growth. Overall, it was proved that methane oxidizing bacteria can efficiently grow using both approaches that were tested to recover nitrogen and nutrients from anaerobic effluent.

### Effect of methane source on SCP production

Methane from different sources was tested to examine whether SCP can be obtained using different sources of biogas as a substitution for natural gas (Fig. 2). The *EDC-recovery* was used to add the nitrogen that was externally enriched with trace elements. High ammonium removal was achieved in all cases: 94% using raw and biologically upgraded biogas, and 97% using pure methane. The highest  $Y_{CH_4}$  was  $0.78 \pm 0.03$  gTSS/gCH<sub>4</sub> using biologically upgraded biogas, followed by raw biogas ( $0.75 \pm 0.08$  gTSS/gCH<sub>4</sub>) and pure methane ( $0.68 \pm 0.013$  gTSS/gCH<sub>4</sub>). Furthermore, the amino acid profiles showed that Aspartic and Glutamic acids were among the most dominant components. These observations are in accordance with Rasouli et al. (2018) who examined the SCP production using *Methylococcus capsulatus*. The results obtained herein proved that biogas can be a good substitution for natural gas for SCP cultivation. The tests highlight that methane oxidizers could grow using both raw biogas and biologically upgraded biogas as carbon source to produce SCP.

## CONCLUSIONS

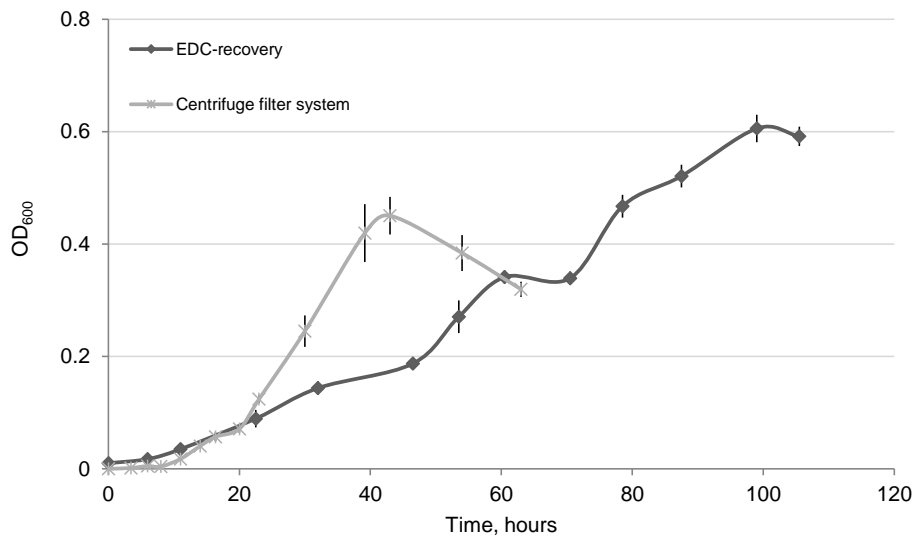
The present study showed that Single Cell Protein can be produced using effluent from anaerobic reactor fed with urban bio-waste. Nutrients and nitrogen were efficiently recovered from anaerobic digestate using an electro dialysis cell. Specifically, *EDC-recovery* led to markedly high (~92%) ammonium removal without establishing non-hygienic conditions for the methane oxidizers. However, the *Centrifuge filter system* could provide all the necessary micro-nutrients to the mixed culture. In addition, biogas and biologically upgraded biogas were used as alternative source of methane than natural gas. The highest biomass yield in terms of methane uptake was achieved injecting biologically upgraded biogas ( $0.78 \pm 0.03$  gTSS/gCH<sub>4</sub>). The present study indicates that the valorization of gas and liquid streams of traditional anaerobic digestion process can support the vision of circular bio-economy through Single Cell Protein production.

## ACKNOWLEDGEMENTS

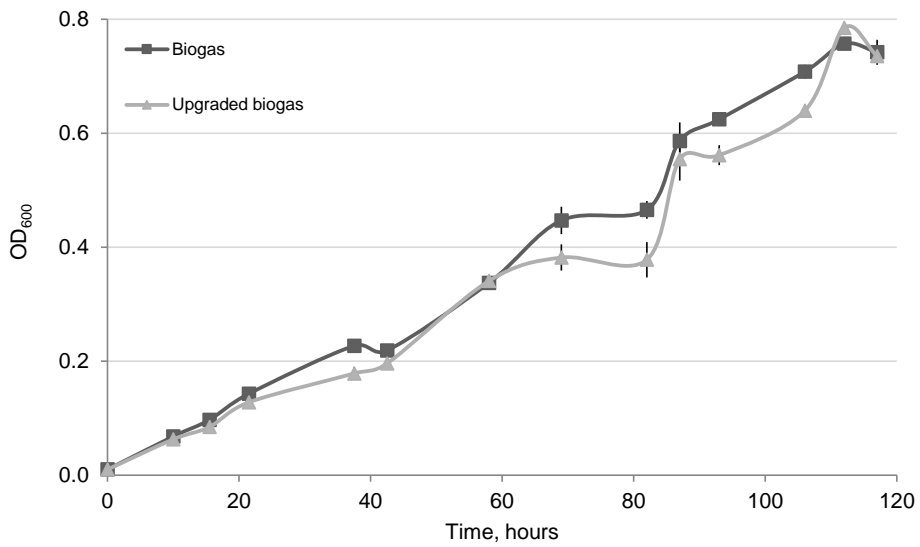
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**Figure 1.** Cultivation of mixed methanotrophic culture using different nitrogen recovery methods



**Figure 2.** Cultivation of mixed methanotrophic culture using different methane sources