Exploring microplate assay as a quick tool to assess the suitability of anaerobic effluents as microalgal growth media

Jai Sankar Seelam [1], Marcella Fernandes de Souza [1], Peter Chaerle [2] , Erik Meers [1]

1. Department of Green Chemistry & Technology, Faculty of Bioscience Engineering, Ghent University, Belgium

2. Department of Biology, Faculty of Sciences, Ghent University, Belgium

GHENT UNIVERSITY

FACULTY OF BIOSCIENCE ENGINEERING

The synergistic collaboration of microalgal and anaerobic bioprocesses for large-scale microbial protein synthesis could potentially improve the sustainability quotient of the agricultural sector. Microalgae can ably recycle the nutrients within Nitrate Vulnerable Zones, where excess of anaerobic effluents, like digestates, cannot be land applied. The availability of nitrogen, phosphorus, potassium and trace metal elements in the digestate promotes microalgal proliferation and intracellular protein accumulation. However, high concentrations may cause growth inhibition. Also, high dry matter content, viscosity and dark color of digestate pose serious problems concerning light penetration, an important parameter for photosynthesis. Thus, pre-treatment of digestate and substrate optimization becomes necessary for better microalgal biocatalysis and algal protein production.

OBJECTIVES

- Studying the suitability of dark-colored liquid fraction of digestate at different concentrations as a substrate for microalgal cultivation
- Investigating a pre-treatment strategy to overcome light penetration and substrate inhibition issues
- Exploring microplate assay as a quick tool for substrate screening and growth optimization

MATERIALS AND METHODS

- Digestate : Liquid fraction (LF) collected from anaerobic digestor with plant-based feedstock (Pittem, Belgium)
- Pre-treatment : Paper-filtration (pore-size : 4 – 11µm)
- Microalgae : Mixed consortium of green algae (Fig 1)
- Cultivation : Mixotrophic with LF as media
- Conditions : White light – 50µmol photons/s/m^2
- Reactor config. : Microplates and Erlenmeyer flasks

Table 1 : Characterization of untreated (raw) and treated (paper-filtered) liquid fraction of digestate

TREATED AND UNTREATED LIQUID DIGESTATE				
Parameter	**Dry Matter (%)**	**pH**	**N (mg/kg)**	**P (mg/kg)**
LF	1.23	7.87	2430	25.31
PLF	1.18	8.34	2370	9.61

- Series of diluted paper-filtered liquid fraction of digestate (PLF) were prepared to assess their light penetrating capacity including turbidity, absorbance & reflectance (Fig 2)
- Particle-size analysis of undiluted PLF was performed using Imaging flow cytometer (Fig 3) to compare their particle size with average microalgal cell size (3 – 10 µm)
- Raw and paper filtered LF were analyzed with Kjeldhal method, ICP-OES and standard colorimetric techniques (Table 1 & 2)

Table 2 : Chemical characterization of undiluted paper-filtered liquid fraction of digestate (in mg/kg)

PLF : MACRONUTRIENTS & TRACE METAL ELEMENTS				
K	**Ca**	**Mg**	**Cu**	**Zn**
1720	35.8	92.1	0.24	1.19
Cr	**Cd**	**Pb**	**As**	**Hg**
0.13	0.02	0.24	0.24	0.02

- PLF (1 – 20% v/v) was used as substrate for growth experiments in microplates (Fig 4) and 100-mL flasks

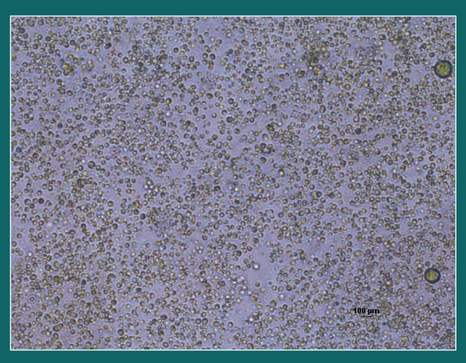


Fig 1 : Consortium of *Chlorella* sp., *Desmodesmus* sp., *Kirchneriella* sp., *Ankistrodesmus* sp. and *Monoraphidium* sp.

Fig 2 : Series of 0-20% v/v samples of paper filtered liquid fraction of digestate. For eg. 10% v/v = 10x diluted with filtered tap water

Fig 3 : ImageStreamX Mark II Imaging Flow Cytometer

Fig 4 : Microplates with working volume of 3 mL

MAIN RESULTS

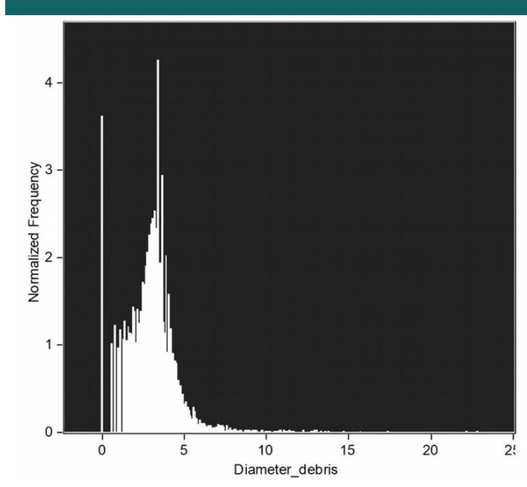
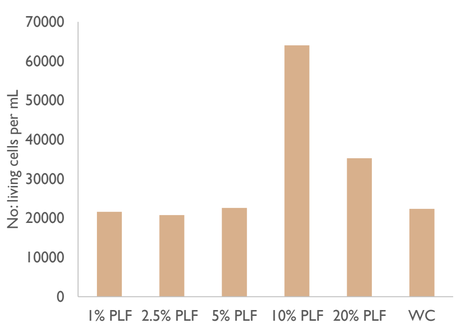


Fig 5 : The particle size distribution of undiluted paper-filtered liquid fraction of digestate. 90% of particles are less than 6.5 µm in diameter and majority fall within the 3.6 – 5.5 µm size interval

- Particles in PLF are similarly sized as the microalgal cells used in this experiment (Fig 5)
- No significant differences in the pH and total nitrogen composition of the liquid fraction were observed following the paper filtration but 62% of the total phosphorus content was lost
- The loss is due to removal of insoluble phosphorus-bound particles (which might not be bioavailable for microalgae)



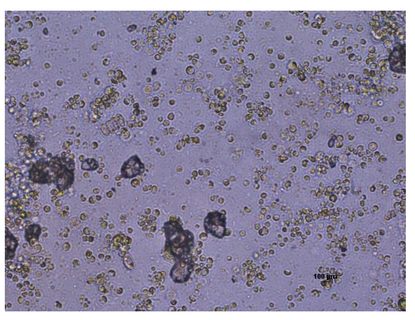


Fig 6 : (L)The distribution of living microalgal cell count in microplates at different PLF concentrations compared to WC medium after 3 days (R) and Microscopic screenshot of biomass of best performing PLF concentration of 10% (v/v) in microplates

- Under non-axenic conditions, growth in 2.5 – 20% (v/v) PLF had comparable living cell count as WC medium (Fig 6(L))
- Increasing trend within 2.5 – 10% PLF but inhibition could be the cause of declining performance at 20%
- After 3 days, maximum cells were visible in both individual and clustered form at 10% (v/v) (Fig 6(R))
- Similar trend of cell count profile was observed in tests with 100-mL flasks after growth period of 14 days (Fig 7)

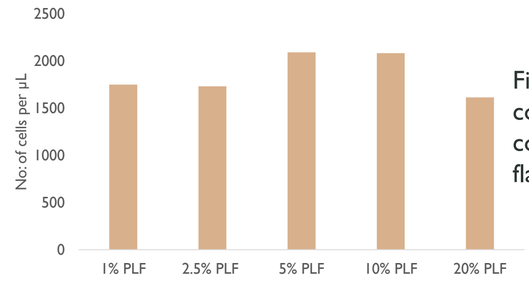


Fig 7 : The distribution of total cell count at different PLF concentrations when grown in flasks for over 14 days

CONCLUSIONS

- Paper filtration could be regarded as a promising technology for pre-treating the liquid fraction of digestate for improved microalgal cultivation
- Substrate screening and growth optimization trials using microplate assays is a cost-effective, space- and time-saving technique
- Low concentration of living cells can be attributed to high N/P ratios in PLF

FUTURE OUTLOOK

- Further experimentation with microplates is required to validate its use for scale-up activities
- Microplate experiments can also be extended to microalgae screening and selection

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European Regional Development Fund

This poster is presented within the framework of the **ALG-AD** project. ALG-AD is financially supported by the European Regional Development Fund provided by Interreg North-West Europe programme in which new technology is being developed to take excess waste nutrients produced from anaerobic digestion of food and farm waste to cultivate algal biomass for animal feed and other value-added products. For more information, visit www.nweurope.eu/ALG-AD