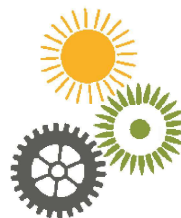


**RRB 2023**  
RENEWABLE RESOURCES & BIOREFINERIES



## **19th International Conference on Renewable Resources and Biorefineries**

**Green gold – Forests for the future**

**31 May - 2 June 2023 • Riga, Latvia**

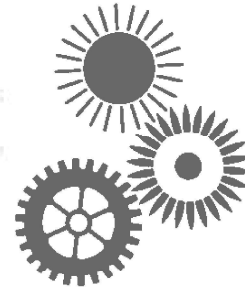


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19<sup>th</sup> International Conference on  
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monitored the keratinase activity of Proteinase K, which degrades the keratin azure substrate and releases soluble products which are observed at 595 nm. In addition to optimising the method, the azure dye standard, Remazol Brilliant Blue R, was used to calibrate the assay and provide information on the kinetics of the keratinase-catalysed reaction. The optimised method was also used to investigate different reaction quenching/work up conditions and filtration was found to be the best method for removing unreacted keratin azure. This assay was then used to investigate the effect of reduction of the keratin disulfide bond on keratinase-catalysed degradation. This method will enable identification of keratinases ideally suited for application in the valorisation of natural wool fibres.

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### BIOCONVERSION OF CO<sub>2</sub> FROM BIOGAS TO BIOMETHANE IN PILOT-SCALE TRICKLED BED REACTOR

**Dana Pokorna<sup>1</sup>, Z. Varga<sup>1</sup>, D. Andreides<sup>1</sup>, O. Vesely<sup>1</sup>, P. Benes<sup>2</sup>, J. Zabranska<sup>1</sup>**

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Biogas contains energetically usable methane and an unavoidable carbon dioxide content. Upgrading biogas to biomethane offers an extension of energy recovery, as biomethane can be distributed through the natural gas grid and more effectively utilized or directly used as the fuel of a vehicle. Although CO<sub>2</sub> removal is commonly used by physicochemical methods, there have been increasing efforts recently to convert it into biomethane using hydrogen. The chemical conversion of CO<sub>2</sub> to methane has disadvantages of requiring high pressure and temperature and an expensive catalyst. In contrast, biological conversion is a process that takes place under much milder conditions. Hydrogenotrophic methanogens can catalyse the biological conversion of CO<sub>2</sub> and H<sub>2</sub> to biomethane at temperatures of 35 °C or 55 °C and pressures close to the atmosphere. In this research, a pilot-scale trickled-bed biofilm reactor was designed as a modular countercurrent three-phase reactor packed with carriers for biomass immobilisation. This arrangement provides a high contact area of the mixture of gases (substrate), liquid, and biomass of hydrogenotrophic methanogenic consortium and by that a high process efficiency. The adapted consortium was immobilised on carriers in the active part in the middle of the reactor with a packed bed volume of 140 L. The H<sub>2</sub> supply and the real biogas as a CO<sub>2</sub> source were located at the bottom of the bioreactor and an industrial membrane disk element provided the gas distribution. H<sub>2</sub> was injected according to the CO<sub>2</sub> content in the biogas in a stoichiometric ratio of 4:1. The bioreactor was operated under thermophilic conditions (55 °C) processing biogas from a municipal wastewater treatment plant (WWTP). During the bioreactor operation for 107 days, the H<sub>2</sub> load gradually increased. The bioreactor was operated stably at a maximum H<sub>2</sub> load of 31.1 L/(L·d) [H<sub>2</sub>; V<sub>bed</sub>] and a biogas load of 15.4 L/(L·d) [biogas; V<sub>reactor</sub>]. At this loading rate, CH<sub>4</sub> production was 12.2 ± 2.4 L/(L·d) and H<sub>2</sub> and CO<sub>2</sub> recovery efficiencies of 98.7 ± 1.0% and 94.0 ± 2.5%, respectively. The CH<sub>4</sub> content of the upgraded biogas was 90.1%.

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### FLUE GAS TREATED MICROALGAE CHLORELLA VULGARIS: BIOCHEMICAL EVALUATIONS AND DEVELOPMENT OF FISH FEED SUPPLEMENT

**Renu Geetha Bai<sup>1</sup>, S. Chandrasekharan Nair<sup>1</sup>, L. Joller-Vahter<sup>2</sup>, T. Kikas<sup>1</sup>**

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Flue gas is a waste by-product released from industries during burning process and it contributes to air pollution and climate change. Based on the fuel composition and combustion parameters, the composition of flue gas can vary. The major constituents are particulate matter, heavy metals, water vapour, and oxides of carbon, sulphur and nitrogen. Due to its environmental impacts, the mitigation of flue gas is crucial factor in achieving carbon neutrality by regulating levels of greenhouse gas emissions. One sustainable approach to address this issue is the utilization of flue gas for algal growth as an alternative carbon source, using the carbon sequestration properties of algae. Therefore, in our study, we investigated the growth and biomass production of the green microalgae *Chlorella vulgaris* in the presence of flue gas. Furthermore, the study evaluated the biochemical changes that occurred in the algal cells in the presence of flue gas via different biomolecules (pigments, carbohydrates, proteins, lipids, enzymes) analysis. Due to the high protein content, the *chlorella* is a preferred aqua feed biomaterial. *Chlorella* harvested after the cultivation process, was used as a fish feed supplement material. The effect of new fish feed studies were conducted on rainbow trout fish models and toxicity profile and nutritional value were analysed, ensuring product quality and safety. Ultimately, the use of flue gas for algal growth can contribute towards achieving the climate neutrality goals of the European green deal, while promoting sustainability and circularity in the process.

Keywords: microalgae, CO<sub>2</sub> sequestration, bioreactors, industrial flue gas, fish feed, biomolecules.

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### A NOVEL BENCH-SCALE PHOTOBIOREACTOR FOR CONTINUOUS CULTIVATION OF MICROALGAE

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There are different types of carbon intensive industries. While some operate with daily intervals, others must be maintained in continuous operation, sometimes for weeks or months. Processes that depend on microbiological activity are usually of the second category, resulting in the continuous production of CO<sub>2</sub> during extensive periods of time. In order to help mitigate climate change, alternative methods of carbon capture into added-value products have been the focus of research.

Autotrophic microalgae cultures can be employed to sequester the carbon present in these streams,



generating new products, while increasing process sustainability. However, to sequester these emissions microalgal bioreactors must also function under continuous constant conditions, requiring photobioreactors (PBRs) that can act as chemostats for long periods of time. Moreover, there is currently a lack of studies and design alternatives using microalgal chemostats. Most works tend to focus on batch assays or semi-continuous processes, presenting different responses depending on the growth stage of the culture, or the time of day.

The present work is centred on the development of a novel continuous bench-scale PBR. This system uses an innovative recirculation concept to combine three different units (retention vessel, photocollector and degasser) that operate as a single autotrophic chemostat, allowing the study of carbon sequestration from a biogenic CO<sub>2</sub>-rich constant air stream. The novel PBR was tested by cultivating the microalga *Haematococcus pluvialis* at different dilution rates (0.1-0.5 d<sup>-1</sup>), while using as sole carbon source an air stream containing ≈0.35 vol% of CO<sub>2</sub> (produced by a coupled heterotrophic bacterial chemostat).

The results obtained revealed that the system could operate as a chemostat, allowing the production of stable cultures with proportional responses to the changes in dilution rates for more than 3 months, reaching a maximum biomass productivity of 183 mg/L/d, with a carbon fixation efficiency of ≈39% at 0.3 d<sup>-1</sup>. This makes the PBR prototype a promising tool to study/optimize integrated heterotrophic and autotrophic continuous processes, or constant sequestration of stable CO<sub>2</sub>-rich streams, making it easier to gather data for future scale-up.

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#### PROSPECTION OF NEW BIOCIDES SOLUTIONS BASED ON OLIVE LEAF EXTRACTS AND ESSENTIAL OILS FOR THE LEATHER INDUSTRY

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In the leather industry, microbial growth leads to biodeterioration, resulting in surface modification, decreased physical-mechanical properties, and stain formation that impairs appearance. This compromises the quality of the final product, resulting in the loss

of commercial value. In general, the used synthetic biocides are toxic and dangerous, both for humans and the environment; being important to replace them to guarantee the sustainability of the leather production process and the final products. This objective can be approached by developing novel preservative strategies and products, preferably based on natural ingredients. In this context, this work proposes the development of eco-friendly solutions with microbicidal potential by combining olive leaf extracts (OLE) with oregano essential oil (OEO). The combinations of oils and extracts can result in synergistic effects improving the antimicrobial capacity against a wide range of microorganisms. Thus, this study aimed to evaluate and compare the potential antimicrobial activity of OLE and OEO extracts, individually or conjugated, against *E. coli* cultures to obtain an environmentally friendly biocide. Two commercial standardized olive leaf extracts were studied, one comprising 20% oleuropein (OLE-O) and the other 20% of hydroxytyrosol (OLE-H). In the first phase, the minimum inhibitory concentration (MIC) was determined in *E. coli* cultures by the microdilution method and colourimetric assay with p-iodonitrotetrazolium chloride (INT). Subsequently, the bacterial reductions were quantified by counting the colony-forming units (CFU) on a plate. The lowest MIC values were achieved for the combinations 1/1 of the OEO with the extracts (OEO/OLE-O and OEO/OLE-H), used at a content of 0.05% and 0.10%, respectively, indicating an increased bacterial inhibition potential and synergistic action between the essential oil and the extracts. The microbial reduction obtained for the conjugated systems was 8 Log<sub>10</sub> CFU/mL for the OEO/OLE-O system, and 7 Log<sub>10</sub> CFU/mL for the OEO/OLE-H, both with a significance of p<0.0001, relative to the control. The obtained results, which will be also extended to other microorganisms, demonstrate the advantages of combining hydrophilic extracts with hydrophobic essential oils, which can be the basis for the development of emulsion-based systems to be applied in the leather industry.

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