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Microalgae as sustainable and potential feedstock for the production of biofuels and fine chemicals

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Keywords: microalgae, chlorella vulgaris, wastewater treatment, biomass valorization, biorefinery.
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Abstract
Microalgae have been identified as a promising and sustainable alternative for wastewater treatment. Their ability to biologically purify wastewater of different origins, using them as a growth substrate, holds great potential as a wastewater treatment method. However, the technology cost is the main factor that limits the industrial scale application. For this reason, it is necessary to introduce a downstream process of the purification process to minimize production costs. The simultaneous use of microalgae for wastewater treatment by the production of high value-added chemical compounds has made this challenge feasible and economically viable. In this work, a detailed analysis of the chemical composition of microalgae (Chlorella Vulgaris) recovered from wastewater treatment was carried out in order to identify the possible prospects for future reuse.

Introduction
As a result of the growing world population and rapid industrialization, the increasing production of municipal wastewater has emerged as one of the major environmental problems. Organic and inorganic pollutants of various origins, including micropollutants and heavy metals, are discharged into water bodies nearby industrial and agricultural activities. The presence of excess nutrients such as nitrogen (N) and phosphorus (P) is the cause of eutrophication of water bodies and the resulting environmental problems. For this reason, there is a need to develop treatment processes that can remove these nutrients before wastewater is discharged. In this sense, the use of algae as a means of bioremediation of wastewater can effectively extract nitrogen and phosphorus from wastewater, keeping dissolved oxygen levels constant and helping to reduce the pathogens and fecal bacteria present in wastewater. In addition, it is also an environmentally sustainable option as it has the ability to convert carbon dioxide into chemicals and useful products, thus helping to reduce greenhouse gas emissions. However, the cost of this technology is the main factor that limits its application on an industrial scale. In order to solve this problem, the development of an integrated approach for the valorization of the produced microalgae would contribute not only to considerably reduce the total costs but to convert the produced biomass "from waste to resource" in accordance with the principles of the circular economy. In this work, a detailed analysis of the chemical composition of microalgae (Chlorella Vulgaris) grown using wastewater as a substrate was carried out.
The identification and quantification of esterifiable lipids, proteins, easy hydrolysable sugars (EHS), lignin and ashes were performed in order to identify the possible prospects of its possible reuse for the production of platform molecules and fine chemicals.

Materials and Methods
Protocol for biomass characterization
About 1 g of dried biomass were startingly dispersed in hexane (20 mL per three times) in order to extract lipid. Hexane phases were collected together and then evaporated until residue was weighted (lipids were even gas-chromatographically determined as methyl-esters by using a procedure of esterification in methanol and HCl Bitonto et al. (2020a). Then, residual solids (RS) obtained from lipid extraction were desiccated at 105 °C and analysed by adapting the National Renewable Energy Laboratory (NREL) procedure to determine carbohydrate structure. Solids were weighed and transferred into a glass balloon with 100 mL of a 4% H_2SO_4 aqueous solution. Then, this suspension was refluxed for two hours, cooled, filtered and diluted in water before analysis with HPAE-PAD for sugar determination (easily hydrolysable sugars, EHS, includes hemicellulose, pectinic sugars and exopolysaccharides) and protein content Bitonto et al. (2020b). Then, the solids recovered from the aforementioned filtration were washed with distilled water and dried at 105 °C for 24 h. This solid was weighed and transferred into a glass tube and kept under suspension with 3 mL of 75% H_2SO_4 at 4 °C for 24 h. After this treatment, water was added to the analyte, achieving a final volume of 100 mL; this solution was again refluxed for two hours. This resulting suspension was cooled, filtered, diluted and analysed for cellulose determination. The RS were thoroughly washed with water, dried at 105 °C for 24 h and weighed. The organic residue was calculated as the difference between this weight and the respective ash obtained after putting the same RS into an oven at 550 °C for 2 h; this difference represents the lignin content. Ashes content was determined directly on the dry sample after thermal treatment after 2 h at 550 °C. The entire protocol for biomass characterization can be summarized in Figure 1.

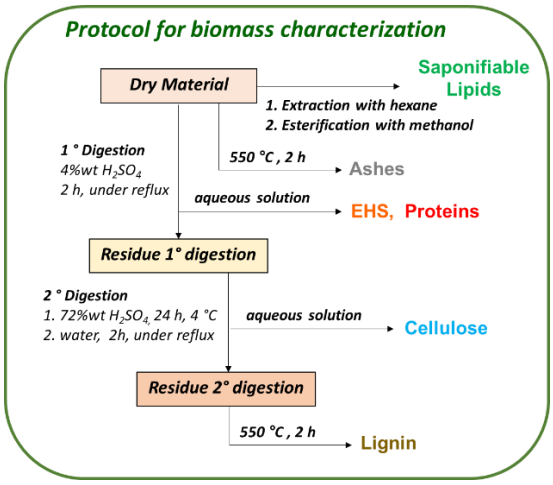

Figure 1. Schematic diagram for biomass characterization.

Identification of carboxylic acids

About 50 µL of water solution deriving from the first digestion of dry biomass (100 mL of a 4% H_2SO_4) were transferred in a vial with insert of a total volume of 250 µL and dried under nitrogen flow at 40 ºC. Then, 50 µL of MTSFA and 50 µL of pyridine were added. The vial was closed and left for 30 min at 40 °C. Finally, this solution was analysed via gas-chromatography (GC–MS).

Results and discussion

Chemical characterization of microalgae

The results of chemical characterization of microalgae (Chlorella Vulgaris) are reported in Tables 1. Overall, the protein content is the main component of this biomass with a value of 556 ± 25 mg/g_{TS}. This suggests a possible use as a biofertilizer for agricultural use. At the same time, the organic acids present 91.3 ± 6.1 mg/g_{TS} (i.e., levulinic, maleic and malonic acids) could be extracted and used for the production of biopolymers and fine-chemicals (ethyl levulinate, γ-valerolactone). Finally, esterifiable lipids have also been identified (74.3 ± 2.1 mg/g_{TS}) that could be used for biodiesel production.

Table 1. Chemical composition of microalgae (Chlorella Vulgaris).

Total solids (TS) composition	
Total Lipids	**85.3 ± 3.1 mg/g_{TS}**
Esterifiable lipids (FAMEs)	*74.3 ± 2.1 mg/g_{TS}*
Proteins	**556 ± 25 mg/g_{TS}**
Easy Hydrolysable Sugars (EHS)	**91.3 ± 6.1 mg/g_{TS}**
Arabinose	*3.9 ± 0.2 mg/g_{TS}*
Glucosamine	*5.9 ± 0.2 mg/g_{TS}*
Galactose	*9.4 ± 0.2 mg/g_{TS}*
Glucose	*59.5 ± 0.2 mg/g_{TS}*
Xylose	*0.5 mg/g_{TS}*
Lignin	**49 mg/g_{TS}**
Ashes	**56 mg/g_{TS}**
Other compounds (i.e. carboxylic acids)	**162.4 ± 31.1 mg/g_{TS}**

Conclusions

Microalgae (Chlorella Vulgaris) deriving from wastewater treatment was characterized in order to determine its potential to be valorised as potential source of biofuels and biochemicals. The high content of proteins and organic acids suggests the main use for the production of biofertilizer and biopolymers, respectively, reducing the total costs of wastewater treatment with the obtaining of products with high added value.

Acknowledges

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Microalgae as sustainable and potential feedstock for the production of biofuels and fine chemicals

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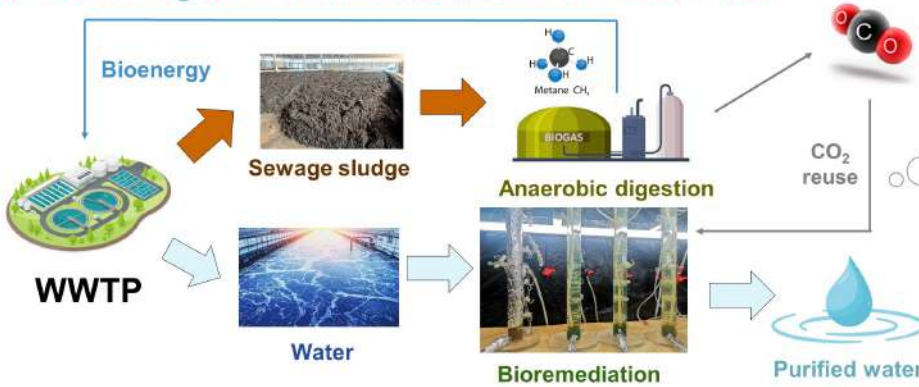
Introduction

As a result of the growing world population and rapid industrialization, the increasing production of municipal wastewater has emerged as one of the major environmental problems. **Organic and inorganic pollutants** of various origins, including **micropollutants** and **heavy metals**, are discharged into water bodies nearby industrial and agricultural activities. The presence of excess nutrients such as **nitrogen (N) and phosphorus (P)** is the cause of eutrophication of **water bodies** and the **resulting environmental problems.** For this reason, there is a need to develop treatment processes that can remove these nutrients before wastewater is discharged.

"The problem of wastewater management"

In this sense, the use of algae as a means of bioremediation of wastewater can effectively extract nitrogen and phosphorus from wastewater, keeping dissolved oxygen levels constant and helping to reduce the pathogens and fecal bacteria present in wastewater

"The use of algae for bioremediation of wastewater"



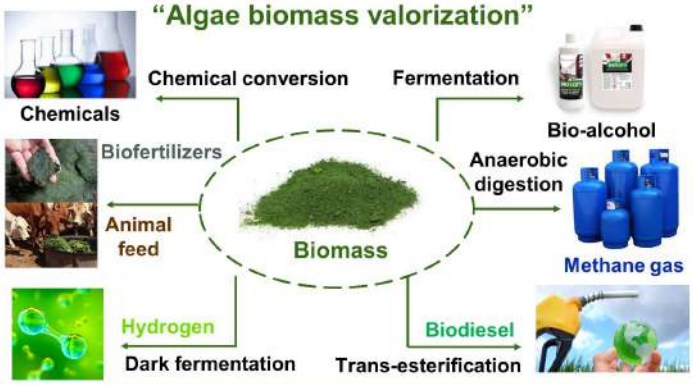
In addition, it is also an **environmentally sustainable option** as it has the ability to convert carbon dioxide into chemicals and useful products, thus helping to reduce **greenhouse gas emissions**

However, **the cost of this technology** is the main factor that limits its application on an **industrial scale**

Aim of work

In this work, a detailed analysis of the **chemical composition of microalgae (Chlorella Vulgaris)** grown using **wastewater** as a substrate was carried out. The identification and quantification of **Total Lipids, Proteins, Easy Hydrolysable Sugars (EHS), Lignin, Ashes** and **Carboxylic Acids** were performed in order to identify the possible prospects of its possible reuse for **the production of platform molecules and fine chemicals**

"Algae biomass valorization"



Results and discussion

Chemical characterization of microalgae "Chlorella Vulgaris"

Total solids (TS) composition	
Total Lipids	85.3 ± 3.1 mg/g_{TS}
Proteins	556 ± 25 mg/g_{TS}
Easy Hydrolysable Sugars (EHS)	91.3 ± 6.1 mg/g_{TS}
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Lignin	49 mg/g_{TS}
Ashes	56 mg/g_{TS}
Other compounds (i.e. Carboxylic Acids)	162.4 ± 31 mg/g_{TS}

The protein content is the main component with a value of 556 ± 25 mg/g_{TS}. This suggests a possible use as a **biofertilizer** for agricultural use. At the same time, **the Carboxylic Acids present 162.4 ± 31 mg/g_{TS} (i.e., levulinic, maleic and malonic acids)** could be extracted and used for the production of **Biopolymers and fine-chemicals**

biofertilizers and **biopolymers**

Conclusions

Microalgae (Chlorella Vulgaris) deriving from **wastewater treatment** was characterized in order to determine its potential to be valorized as potential source of biofuels and biochemicals. The high content of **Proteins** and **Carboxylic Acids** suggests the main use for the production of **Biofertilizers and Biopolymers**, respectively, reducing the total **costs of wastewater treatment** with the obtaining of products with high added value.

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