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Poster session

81 Guocheng Wang, Haoyu Xiao, M. Sieradzka, Zuzanna Prus, M. Wilk, Yingquan Chen, Haiping Yang, Jiawei Wang, Yang Yang, A. Magdziarz
Experimental investigations of polyethylene hydrothermal treatment towards high hydrocarbons

82 Á. García-Ruiz, J. A. Rodríguez-Liébana, Sofía Jurado-Contreras, F. J. Navas-Martos, P. González-Torres, M.Dolores La Rubia
Liquid hot water as a pre-treatment in the fractionation of cotton and sunflower stalks

83 D. Ciolkosz, A. Bialowiec, T. Causer, L. Nunes, W. Rasac, J. Vasco
Organic Compounds and Biorefinery Biochar: Towards Optimization of Contaminant Control and Treatment

84 M.C. García Vargas, A. García Arreola, I. Gómez-Cruz, F.J. López Hernández, M. M. Contreras, J.M. Romero-García, E. Castro
Lean Manufacturing in the Conversion of Avocado Waste into Biofuels

85 M.M. Vainio, S.E. Rasi
Anaerobic processing of food waste and used cooking oil

86 S. Narra, C. M. Lex, M. M. Narra
Hybridization – Unlocking potentials by complementing each other

87 A. Karagianni, A. Zourou, A. M. Tavlaridi, D. Katerinopoulou, A. Stoumpidi, I. Kitsou, A. Tsetsekou, G. Kiriakidis, K.V. Kordatos
Photocatalytic Applications of TiO_2@Carbon Dots Core-Shell Nanocomposite Decorated with Ag NPs

88 A. Escobar, L. Montes, J.A. Vázquez, A. Franco-Uría, R. Moreira
Oleogels from fish discards (Scomber japonicus) obtained by emulsion template method using hydroxypropyl methylcellulose as structuring agent

89 E. Pareja-Sánchez, J. Lechuga Puñal, A.J. García-Moreno, P. Domouso, V. Ochoa Esteban, R. García-Ruiz
Carbon Sequestration in Mountainous Olive Groves and its Potential in the CO_2 Credit Market at the Cooperative Level

90 S. López-Rayo, A. M. Stefan, J. M. Lozano-González, B. Mayans, C. García-Delgado
Composting of Algae Residues from Mar Menor Lagoon: A Sustainable Organic Amendment for Enhancing Lactuca sativa Growth in Saline Calcareous Soils

91 A. Hejna, M. Barczewski, J. Andrzejewski, J. Aniśko, J. Korol
Upcycling of wood and polyurethane foam wastes into composite materials

92 A. Imre-Lucaci, V.-C. Sandu, F. Imre-Lucaci, S. Fogarasi
Mediated electrochemical recovery process of copper from waste printed circuit boards

93 Á. Galán-Martín, S. Bueno-Rodríguez, R. Cabrera-Jimenez, G. Guillén-Gosálbez, I. Romero-Pulido
Innovative Biochar-Based Ceramic Materials: Insights from Coupling Lab-Scale Prototypes with Prospective Life Cycle Assessment

94 I. González García, B. Riaño
Guidelines for the selection of nutrient recovery technologies in wastewater treatment plants for bio-based fertilizers production using PROMETHEE methodology

95 L. di Bitonto, A. Li, C. Pastore
Development of novel methodologies for producing volatile fatty acids from municipal sewage sludge

96 S. Grozdanova, I. Trendafilova, M. Popova
Synthesis of mesoporous silicas with biomass derived carboxylic acids as templates: mechanism of formation and templates reuse

97 S. Viciana, D. Franco, A. Franco-Uría, J.A. Vázquez, J.Sineiro
Harnessing of fishing fatty discards to obtain structured oleogels with chitosan and vanillin

98 P. Lombardi, S. Barbero, S. Fiore, I. Orlandella, F. Rovera, E. Todella
Multidimensional sustainability assessment of circular orange peel waste management

Development of novel methodologies for producing volatile fatty acids from municipal sewage sludge

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Keywords: sewage sludge, anaerobic fermentation, volatile fatty acids, circular economy
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Abstract
With the increasing demand for sustainable waste management and renewable energy sources, converting organic waste into valuable biochemicals is a promising solution that can reduce environmental impact and contribute to a circular economy. In this study, the production of volatile fatty acids (VFAs) from municipal sewage sludge by anaerobic fermentation processes is investigated, specifically using cellulose-enriched sludge obtained by dynamic sieving. Without any pretreatment of the starting biomass and under very mild reaction conditions (35 °C, 14 days), a significant increase in the production of VFAs from municipal sewage sludge was observed (6.8 g/L) with acetic and propionic acids being the main compounds. The results underline the potential use of by-products from wastewater treatment as a resource to promote a circular economy approach to waste management.

Introduction
Managing sewage sludge produced during wastewater treatment is a significant environmental challenge. With increasing urbanization and industrial activity, the amount of sewage sludge produced has increased dramatically, requiring effective disposal and management strategies. Conventional methods of sewage sludge disposal, such as landfilling or incineration, raise concerns about their environmental impact and sustainability (Gusiatin *et al*, 2024). In recent years, the potential to utilize sewage sludge as a resource has gained attention, mainly through anaerobic fermentation processes that convert the organic matter into volatile fatty acids (VFAs). These compounds can serve as valuable intermediates for various applications, including bioplastics, biofuels and chemical feedstocks. Investigating the anaerobic digestion of cellulosic sewage sludge (obtained through sieving of urban wastewater) to produce VFAs addresses waste management issues and promotes a circular economy by converting waste into valuable products. This work investigates the production of VFAs from cellulosic municipal sewage sludge through anaerobic fermentation processes, establishing the groundwork for a more in-depth exploration of the methods, benefits and challenges associated with producing valuable compounds from waste, thereby promoting sustainable solutions.

Materials and methods
Reagents and instruments
All reagents and solvents used in this work were of analytical grade (≥99%) and used directly without purification or treatment. VFAs were quantified by high-performance liquid chromatography (HPLC) using a JASCO instrument with an AS 2055 autosampler, a UV-150 detector (detection wavelength 210 nm) and a Hi-Plex H column (300 mm, 4 mm). The column was thermostatically controlled at 328 K using a flow rate of 0.6 mL min^{-1} of an aqueous 10 mM H_2SO_4 solution as the mobile phase.

Substrate and inoculum
Cellulosic sewage sludge from the wastewater treatment plant of Vernole (6.000 population equivalents, PE, Italy) was used as the substrate for this study. Sewage sludge from the anaerobic digester of Bari Ovest (230.000 PE, Italy) served as inoculum. Samples were treated immediately to avoid prolonged storage (up to 48 h at 4 °C) and analyzed for the determination of total solids (TS), esterifiable lipids, easily hydrolyzable carbohydrates (EHC), proteins, cellulose, lignin and ash content (di Bitonto *et al*, 2020). The chemical properties of the substrate and the inoculum are listed in Table 1. Each determination was carried out in triplicate to calculate the average value and standard deviations. The inoculum was stabilized at 25 °C and atmospheric nitrogen pressure for 20 days under stirring (250 rpm) and stored at 4 °C before the use.

Anaerobic fermentation tests
In a 250 mL Pyrex glass reactor, 1 g of dry sludge was placed with 50 g of inoculum under nitrogen flow. The system was closed and placed in a thermostatic bath under stirring (250 rpm) at 35 °C for 14 days. At the end of the process, the reagent mixture was centrifuged. The aqueous solution was filtered through a 0.45 μm hydrophilic PTFE filter, diluted 1:10 and analyzed by liquid chromatography to determine the VFA content.

Table 1. Chemical properties of the substrate and inoculum used for the anaerobic fermentation tests.

Chemical properties	**Inoculum**	**Substrate**
Total Solids (TS, %wt)	3.0 ± 0.1	35.9 ± 1.4
TS composition	**$\%wt_{TS}$**	**$\%wt_{TS}$**
Esterifiable Lipids	0.5	4.5 ± 0.3
EHC	4.6 ± 0.1	6.5 ± 0.1
Proteins	11.3 ± 0.5	8.0 ± 0.6
Cellulose	1.8	48.8 ± 1.2
Lignohumic-like compounds	13.8 ± 0.9	15.4 ± 1.0
Ashes	64.8 ± 1.8	13.2 ± 0.7

Analysis of results

Anaerobic fermentation of sewage sludge for the production of VFAs

Figure 1 presents the main results related to the anaerobic fermentation of sewage sludge for the production of VFAs. The reaction was carried out at 35 °C for 14 days, using a weight ratio starting biomass to inoculum of 1:50. At the end of the process, a total production of VFAs of 6.8 g/L was observed with acetic and propionic acids being the predominant compounds (48.5%wt and 22.1%wt, respectively).

VFAs	**g / L**
Acetic acid	**3.3**
Propionic acid	**1.5**
Butyric acid	**0.1**
Valeric acid	**0.9**
Hexanoic acid	**1.0**
Total	**6.8**

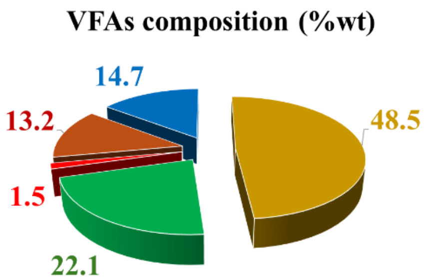

Figure 1. Analysis of VFA production from the anaerobic fermentation of sewage sludge.

In addition, in the end, valeric acid and hexanoic acid production were observed (0.9 g/L and 1.0 g/L, respectively), resulting from the chain elongation process catalyzed by the bacteria in the inoculum. These compounds have a helpful application in industry, especially for producing biosolvents and high-added-value compounds (e.g. bioplastics, biofuels, additives) (D'Ambrosio *et al*, 2024). Further studies are being conducted to promote the chain elongation process of VFAs and to obtain hexanoic acid as the main product while developing innovative extracting procedures.

Conclusions

This work proposed the production of VFA by anaerobic fermentation of municipal sewage sludge. Converting waste into high-value compounds reduces the total waste for disposal and promotes the efficient use of available resources, which aligns with the principles of the circular economy.

Acknowledges

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DEVELOPMENT OF NOVEL METHODOLOGIES FOR PRODUCING VOLATILE FATTY ACIDS FROM MUNICIPAL SEWAGE SLUDGE

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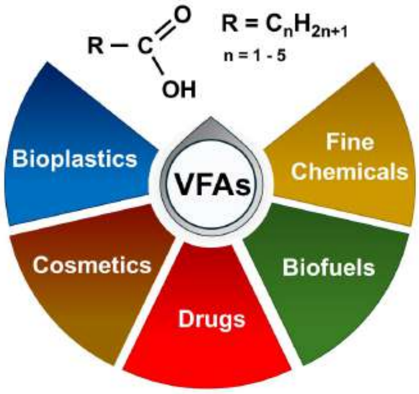
1 INTRODUCTION

The **management of sewage sludge** from wastewater treatment poses a major **challenge for the environment**. With increasing urbanization and growing industry, sludge production has increased, requiring effective disposal strategies. **Conventional methods such as landfilling and incineration** pose environmental and sustainability problems. Recently, the utilization of sewage sludge as a resource, through anaerobic fermentation to produce **Volatile Fatty Acids (VFAs)**, has gained attention. These **VFAs** can then be used as raw materials for manufacturing **fine chemicals**, **biofuels**, **drugs**, **cosmetics** and **bioplastics**.



This work investigates the **production of VFAs** from **municipal sewage sludge** through **anaerobic fermentation processes**, establishing the groundwork for a more in-depth exploration of the methods, benefits and challenges associated with the **production of valuable compounds from waste, thereby promoting sustainable solutions.**

2 MATERIALS AND METHODS

Substrate and inoculum

Cellulosic sewage sludge from the wastewater treatment plant of Vernole (6.000 PE, Italy) was used as the substrate for this study. Sewage sludge from the anaerobic digester of Bari Ovest (230.000 PE, Italy) served as inoculum.

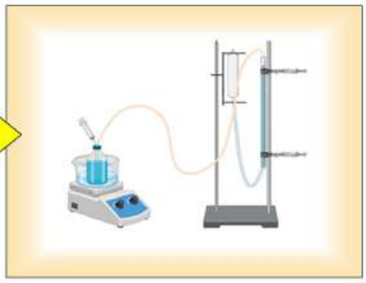
Anaerobic fermentation tests

In a 250 mL Pyrex glass reactor, 1 g of dry sludge was placed with 50 g of inoculum under nitrogen flow. The system was closed and placed in a thermostatic bath under stirring at 35 °C for 14 days.

Chemical properties	Substrate	Inoculum
Total Solids (TS, %wt)	35.9 ± 1.4	3.0 ± 0.1
TS composition		
Esterifiable Lipids	4.5 ± 0.3	0.5
EHC	6.5 ± 0.1	4.6 ± 0.1
Proteins	8.0 ± 0.6	11.3 ± 0.5
Cellulose	48.8 ± 1.2	1.8
Lignohumic-like compounds	15.4 ± 1.1	13.8 ± 0.9
Ashes	13.2 ± 0.7	64.8 ± 1.8

Reaction conditions

N_2, 35 °C
280 rpm
14 days



Fermentation apparatus

Analysis of chemical products

- Centrifugation reagent mixture 4000 rpm
- Recovery and filtration aqueous solution
- VFAs analysis by liquid chromatography

3 RESULTS AND DISCUSSION

A total production of VFAs of 6.8 g/L was observed with **acetic** and **propionic** acids being the predominant compounds (**48.5%wt** and **22.1%wt**, respectively).

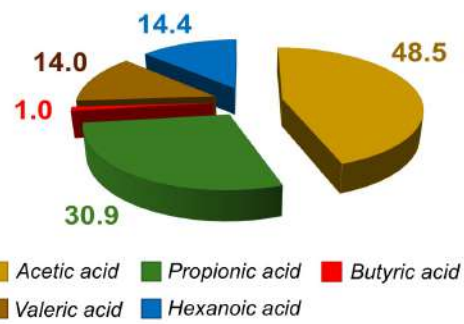
In addition, the production of **valeric** and **hexanoic** acids was achieved (**0.9 g/L** and **1.0 g/L**, respectively), resulting from the process of chain elongation catalysed by the bacteria present in the inoculum.

These compounds are used in industry to produce biosolvents, bioplastics, biofuels and additives.

Analysis of VFAs composition (%wt)

VFAs	g / L
Acetic acid	3.3
Propionic acid	1.5
Butyric acid	0.1
Valeric acid	0.9
Hexanoic acid	1.0
Total	**6.8**

Total COD = 10 g / L



Further studies are being conducted **to promote the chain elongation process of VFAs** and to obtain **hexanoic acid** as the main product

4 CONCLUSIONS

This work proposed **the production of VFA by anaerobic fermentation** of **municipal sewage sludge**. Converting waste into high-value compounds reduces the total waste for disposal and promotes the efficient use of available resources, which aligns with **the principles of the circular economy**.

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