

# Impact of Biochar Amendment on Pilot-Scale Anaerobic Digestion of Agro-Industrial Waste with Digestate Recirculation

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**Abstract** This study evaluated the impact of biochar amendment on the performance of a pilot-scale anaerobic digester treating a mixture of olive pomace mill wastewater, cheese whey, and chicken manure under digestate recirculation. The 180 L mesophilic reactor operated for 160 days at a hydraulic retention time of 20 days, with biochar added to the feedstock at 10 g L<sup>-1</sup>. Process monitoring included measurements of biogas production rate, methane content, and key physicochemical parameters (pH, TS, VS, sCOD), while microbial community dynamics were assessed via 16S rRNA amplicon sequencing and real-time PCR. Biochar addition led to a modest reduction in biogas production rate (from 290.7 ± 17.5 L d<sup>-1</sup> to 273.8 ± 14.3 L d<sup>-1</sup>), but enhanced methane content from 70 % to 73 %. Notably, the relative abundances of *Methanotrix* and *Methanospirillum* increased substantially (from 47.1 % to 74.1 % and 2.2 % to 5.6 %, respectively), indicating strengthened direct interspecies electron transfer pathways. These findings demonstrate that biochar can selectively enrich syntrophic methanogenic consortia and improve methane yield, offering a promising strategy for optimizing pilot-scale anaerobic digestion of agro-industrial residues.

**Keywords:** biochar, anaerobic digestion, DIET, methane enhancement

## 1. Introduction

Anaerobic digestion is an established technology for waste and wastewater treatment converting organic matter into biogas, a renewable fuel that could be used for electricity and heat production. It is estimated that more than 130,000 commercial biogas plants are in operation all over the world while in EU, biogas production was about 31 billion cubic meter in 2015, which will be reached up to 70 billion cubic meter in 2030 (Xue et al., 2020). However, the biogas remains an under-utilized renewable resource for energy production contributing only 0.2–0.4 % to worldwide production of electricity (Jiang et al., 2012).

Different types of technique and operation could improve the performance of the process. For instance, the addition of electrically conductive materials such as activated carbon, biochar, carbon cloth and graphite in anaerobic

digesters could a) reduce lag time for methane formation between 10–75 %, b) increase methane formation rate by 79–300 %, and c) improve the resistance of anaerobic digesters to low pH condition and high ammonia concentration (Park et al., 2018).

The critical point of those interventions is to verify the benefits that exist, not only in lab-scale experiments, but also at pilot-scale. In this context, the scope of this work was to examine the effect of biochar addition into a pilot-scale anaerobic digester treating olive pomace mill wastewater, cheese whey, and chicken manure operating under digestate recirculation.

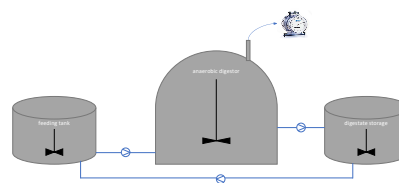


Figure 1. Schematic presentation of pilot unit

## 2. Materials and methods

The pilot plant (Figure 1) consisted of a feed tank (150 L), a mesophilic anaerobic digester (180 L), and a digestate storage tank (150 L). The system operated for 160 days treating a mixture of olive pomace mill wastewater, cheese whey and chicken manure. The hydraulic retention time (HRT) in the anaerobic digester was 20 d. Biochar was added in the feeding tank at a concentration of 10 g L<sup>-1</sup>. Samples were taken from the inlet and the outlet of the digester and were analyzed for pH, TS, VS, sCOD. The volume and the composition of produced biogas was also determined using a gas flow meter (Ritter, Germany) and portable biogas analyzer (GEOTECH, UK), respectively. Microbial community analysis was carried out by means of 16S rRNA amplicon sequencing and real-time PCR.

### 3. Results and discussion

Biochar addition in the anaerobic reactor had as a result a decrease of biogas production rate from  $290.7 \pm 17.5$  L/d to  $273.8 \pm 14.3$  L/d, as shown in Figure 2. On the other hand, methane content increased from 70 % to 73 %. According to literature, biochar generally enhances biogas production (Sunyoto et al., 2016).

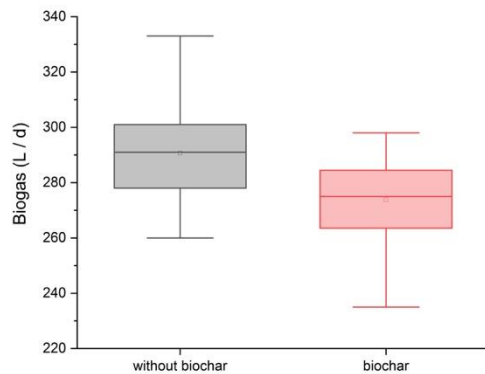


Figure 2. Biogas production range

Lab-scale experiments in the past found that biochar addition could increase biogas production rate from ~ 15 % to 500 % (Qiu et al., 2019). In this pilot-scale study an increase of about only 4.3 % was recorded.

The relative abundance of *Methanothrix* and *Methanospirillum* in the digester increased from 47.1 % and 2.2 % before the addition of GAC to 74.1 % and 5.6 % after the addition of biochar, respectively. The enrichment of these genera may be attributed to DIET that is established via biochar (Abid et al., 2023; Gao et al., 2024).

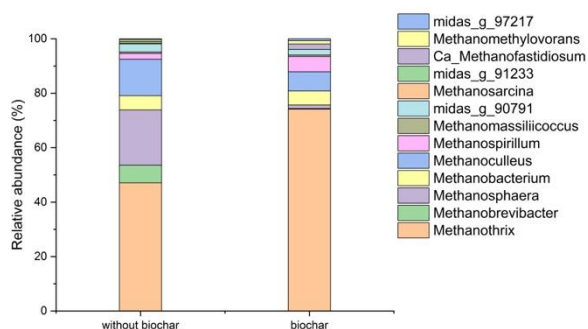


Figure 3. Genera classification of microbial communities

### 4. Conclusion

The addition of biochar in the pilot-scale anaerobic digester treating olive pomace mill wastewater, cheese whey, and chicken manure decreased biogas production rate and increased methane content. The enhanced methane production might be attributed to the enriched *Methanothrix* and *Methanospirillum*. Biochar addition may promote the electron transfer between the syntrophic bacteria and methanogens.

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