Tomato plant waste as a new substrate in biogas fermentation

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Fossil fuels are the most common energy sources all over the world. Their extensive use has led to a massive pollution of the air, water and soil engendering significant critical environmental impacts such as greenhouse effect intensification which induces profound climate change (Fanelli, 2014). Moreover, these non-renewable energy sources are getting exhausted and available only for a quite limited time period. Therefore, finding novel, renewable environmental friendly energies to replace the fossil fuels is among the most urgent and challenging scientific, economic and social concern. Biomass is an intermediate energy store of the Sun energy which can be converted to various biofuels such as biogas, bioalcohols or biohydrogen. Biogas is one of the most prevalent biofuels produced worldwide (Demirel, 2008).

Huge amount of raw plant material is produced every year on a global scale, giving us the opportunity to make a good use of it. Tomato plant (*Lycopersicon esculentum*) is cultivated widely as a vegetable crop this practice produces considerable tomato plant waste (plant stem and leaf in this case). The tomato industry generates quasi 320.000 tons of tomato residue yearly (José M., 2008). The management of these wastes raises diverse environmental and economic problems.

In this study we wanted to examine the biomethane potential of tomato plant waste and its effect on the microbiome of an anaerobic digestion system. Our aim is to use the tomato plant waste for biomethane production and use the fermentation effluent as a fertiliser in the agriculture since tomato plant waste has five times higher nitrogen content than the widely used biogas plant substrate, the corn stover. Besides, the corn stover has more than 10% higher carbon content compared the tomato plant waste. However, our data disclosed that the tomato plant waste had the same biomethane potential as the corn stover. In addition, we carried out 16S metagenomic analysis for monitoring the possible changes in the microbiome.

Materials and Methods

1. Fiber analysis

FIWE 3 Fiber Analyzer (VELP Scientifica, Usmate, Italy) was used for determination of the substrates' fiber composition according to the Van Soest method.

2. Determination of C/N ratio

To determine the C/N ratio of the substrates, an Elementar Analyzer Vario MAX CN (Elementar Group, Hanau, Germany) was used.

3. Gas chromatographic analysis

The methane content was measured daily with Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, United States).

4. Volatile fatty acids (VFAs) analysis

The analysis of different VFAs were performed with VWR Hitachi Chromaster High Performance Liquid Chromatography (VWR International Ltd., Lutterwoth, United Kingdom).

5. DNA extraction and metagenomic analysis

DNA extraction was carried out with the Quick-DNA Fecal/Soil Microbe Kits (Zymo Research Corporation, Irvine, USA) according to the manufacturer's instructions. The sequencing was run on Illumia MiSeq platform (V3 Kit) and the results were analyzed with QIIME 2 program.

Results

Batch fermentation

For the determination of tomato plant waste's biomethane potential, we carried out batch fermentation according to VDI 4630 standard. The inoculum was collected freshly from a mesophilic biogas plant which is operating with pig slurry and maize silage. In our study we used the following substrates: corn stover, tomato plant waste and mixed plant material. The mixed substrate contained corn stover and tomato plant waste in the following mix ratio 0.7:0.3. The biomethane production was measured every day and after the measurement we flushed the "bioreactors" with nitrogen gas. Based on our experiments tomato plant waste can be a good substrate in biogas

plant in spite of its elevated nitrogen content. The biomethane potential of tomato plant waste is very similar to the most commonly used substrate, corn stover as well as the mixed substrate. Interestingly, the methane production is faster from tomato plant material during the fermentation. To explain this phenomenon, we carried out fiber analysis on the various substrates.

Fiber analysis and organic acids detection

According to our results, it could be concluded that the tomato plant waste contains more accessible compounds than the corn stover and it causes a faster methane production. Our HPLC measurements confirmed this result; the fermentors fed with tomato plant waste had higher volatile organic acid concentration at the beginning of the fermentation. These soluble acids could be converted to biogas more efficiently.

DNA extraction and metagenomics analysis

We also followed the microbial composition in the fermentors. Samples were taken on the 0., 3., 6., 11 and 30. days for metagenomics analysis from all substrates (sludge as a control, corn stover, tomato plant waste, mixed plant material). After DNA purification, the library preparations were executed according to the manufacturer's instructions (Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System Guide) except that we have used a modified primer pair for the library preparation for the simultaneous analyses of the group Bacteria and Archaea according to (Takahashi, 2014). Raw sequences were analyzed by QIIME^{TM} (version 1.9.1) (ElNaker, 2018) using published bioinformatics pipelines. Based on the analysis there are several microorganism groups (both Archaea and Bacteria) whose relative abundances changed when we used tomato plant waste. E.g. *Fibrobacteraceae*, *Cloacimonadacea*, *Marinilabiliaceae* occurred with lower abundance in the fermentors which were fed with tomato plant waste while *Lentimicrobiacea*, *Rikanellacea*, *Methanocullaceae* had higher abundance in these reactors compared to the fermentations performed with corn stover.

Conclusion

Tremendous amount of tomato plant waste is annually produced all over the world which can be a promising substrate in biogas fermentation. The batch fermentation of this substrate resulted in similar biomethane yield as the widely used substrate, corn stover. Tomato plant wastes has substantially lower C/N ratio than corn stover and this value decreases during biogas production. Therefore, the fermentation effluent can be a promising bio-fertiliser with relatively high nitrogen content.

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