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MICROALGAE PRODUCTION FROM DIGESTATE OF DRY ANAEROBIC DIGESTION OF ORGANIC WASTE SEQUESTERING CO2 FROM AIR

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The circular economy approach was exploited in this study to exploit a waste produced from the anerobic digestion of organic waste, such as digestate to the final production of microalgae. This biomass production was realized sequestering CO_2 from air.
The digestate originated from a dry anaerobic digestion pilot plant of the OFMSW located at the Fondazione Edmund Mach (S. Michele a/A, Trento, Italy), details are reported elsewhere [1,2]. The microalgae growth experiment was performed in a 15 L laboratory scale system. 13 L of diluted digestate were incubated; to ensure aerobic conditions, the digestate was continuously fluxed with air for 5 days. After this incubation, algae inoculum was added to digestate. A common green algae Chlorella vulgaris (strain Chlorella cf vulgaris K-1801 SCAAP-DK) was used as inoculum. This microalgae was initially grown on Algal Broth culture medium 25 °C with a 14:10 light: dark cycle with 50 µmol m^{-2} s^{-1} measured at the culture surface using a Quantum PhotoRadiometer (Delta Ohm srl, Caselle di Selvazzano, PD, Italy). Algal cells were harvested by gentle centrifugation and washed twice with distilled water before inoculation to insure no nutrient carryover. The microalgae were grown in controlled conditions in 15 L laboratory scale system. The temperature was maintained at 25 °C and the 12:12 dark/light cycle (7.00 a.m. – 7.00 p.m.) was guaranteed with Lucalox psl (LU400W/PSL/T/E40) at 400 watts with 65-µmol m2-1sec-1 of photosynthetically active radiation (PAR). The algae inoculum was diluted in 2 L of distilled water and then added to the digestate medium for a final concentration of 20% v v^{-1}. Two trials were performed: Laboratory Scaled digestate (LSd -without CO_2 addition) and LSCO2 (with CO_2 addition). The control consisted of flasks with tap water without nutrient addition (Cw). The supply of carbon dioxide was controlled via CO_2 mass flow (bronkhorst, NL) to maintain the CO_2-to-ambient air ratios of 0.035% v/v. Moreover, the flux of CO_2-air guaranteed a continuous mixing of the medium. The feed flow gas rate was 0.2 vvm of the enclosed laboratory scale system ($LSCO_2$), while the test without CO_2 addition (LSd) was performed in the open laboratory scale system and mixed without aeration and CO_2 supply. Microalgal growth was determined through cell counts using a Fuchs-Rosenthal chamber slide and counting through microscope at 10 X and 20 X magnifications to determine cell concentration. Every three days, 0.5 ml of growth medium was sampled diluted with 3 ml of distilled water and fixed with Lugol. To calculate biovolume, at least 400 algal cells were counted, and separated by the size of the cells. The optical density usually used to evaluate growth could not be applied in our case due to the color of digestate and to potential changes during the experiment.

Digestate used and characterization

The dilution of digestate (1:70) decreased ammonia concentration to 80 mg L^{-1}, lower than the level considered toxic (100 mg L^{-1}) for microalgal growth [3]. The N:P ratio (calculated on ammonia and phosphate concentration) was about 15. High levels of ammonia have potential toxic effects for microalgae, while low P concentrations could have limiting effects on microalgal growth [4]. Our TS digestate concentration was about 2.85 g SS L^{-1} and was higher than the TS concentration used in the microalgal growth with diluted liquid phase from anaerobic digestate [5], but our ammonia concentration (Table 1) was lower than 260 mg L^{-1} used in the experiment mentioned above. The digestate from OFMSW could contain potentially toxic heavy metals, but also vitamins and amino acids, which can benefit microalgal growth [6].

Table 1: Digestate characterization after and before pre-treatment for microalgae growth.

		Digestate		Pre-treated digestate after microalgae inoculum addition
pH	--	8.14	--	8.01
TS	g 100 g	29.80	g L^{-1}	4.25
VS	g 100 g	17.56	g L^{-1}	2.51
N tot	mg kg^{1}	10400	mg L^{-1}	160.6
NNH_4	mg kg^{-1}	5120	mg L^{-1}	79.09
NO^{3-}	mg kg^{-1}	--	mg L^{-1}	--
P tot	mg kg^{-1}	1250	mg L^{-1}	19.3
PPO^{4-}	mg kg^{-1}	349	mg L^{-1}	5.38

Chlorella vulgaris growth

Algal growth is directly affected by the availability of nutrients, light and stability of pH, temperature and the initial inoculation density. Our results demonstrate the suitability of raw diluted digestate from OFMWS to grow microalgae. The productivity data (Table 2) were lower than other studies. Xia and Murphy (2016) report biomass productivity and concentration (dry weight) in the range of 0.03 – 0.67 g L^{-1} day^{-1} with an algal production in the range 0.4 – 4.8 g L^{-1} for microalgae cultivated on liquid digestate [7]. In any case, the comparison with microalgal growth from other studies is not straightforward due to the wide range of cultivation conditions that can affect biomass production as shown by Franchino et al., 2016 [8]. The growth rate (Table 2) was calculated for each sample trial at the end of the logarithmic phase, which corresponded to 7 days (from 3 to 10 days for $LSCO_2$ tests and from 7 to 14 days for LSd tests). Specific growth rate (μ) ranged from 0.37 to 0.43 days in trials with digestate and CO_2, while the specific growth rate of the trials with only digestate were lower (0.14). The OMFSW digestate used as a medium for microalgae growth was very turbid and has a high particulate content, which was not removed through solid-liquid separation, as occurs when producing liquid digestate. The high turbidity of the digestate caused by particulate matter is an important issue, although microalgal cultivation can partly reduce the turbidity by removing suspended solids. Because photosynthesis is dependent on the availability of PAR, high turbidity leads to lower PAR and thereby reduces microalgae growth [9]. The lower growth of microalgae in the control sample and LSd trials compared with $LSCO_2$ were also explained by the lack of feed gas and CO_2 enrichment. For this reason, the microalgae tended to settle to the bottom of the photobioreactor where particulate matter and mutual shading (due to initial very high algae concentration) reduced the growth of Chlorella. The increase of microalgal density leads to increased O_2 consumption due to algal dark respiration caused by mutual shading. This agrees with our results, the maximum algal production in the $LSCO_2$ tests were 2.84 dry g L^{-1}.

Table 2 - Chlorella vulgaris growth parameters and CO_2 fixation rate and CO_2 utilization efficiency assessed based on the productivity parameters detected.

	Cell	Biovolume	P	μ	CO_2 fixation rate	CO_2 utilization efficiency
	n° cell mL^{-1}	mm^3 mL^{-1}	dry g L^{-1} day^{-1}	day^{-1}	g CO_2 L^{-1} day^{-1}	%
LS_D	2.91 10E0^7	4.97	0.036	0.12	0.07	-
LS_{CO2}	6.22 10E0^7	23.31	0.151	0.41	0.31	13.73

Conclusion

The experimental results of Chlorella vulgaris growth was 151 dry mg L^{-1} day^{-1} in laboratory scale system with CO_2 biofixation rate 310 mg CO_2 L^{-1} day^{-1}. The digestate could be consider as a nutrient supplier.

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