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SIMULATED SOFC EXHAUSTS AND THEIR FIXATION ON *CHLORELLA VULGARIS*: STUDY ON AFFECTING PARAMETERS

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ABSTRACT

To curtail greenhouse gas emissions local and distributed energy systems should be fed by renewable and high efficiency fuels. Anaerobic digestate of organic waste from biogas production can be adopted as a substrate, coupled to Solid Oxide Fuel Cell (SOFC) exhausts, for biomass culture. This biorefinery concept can be exploited towards the complete integration of a waste treatment plant. The work concern the preliminary tests assessed to identify the condition to obtain microalgal growth on dry digestate from anaerobic digestion of organic waste and CO_2 remaining after power and heat production by fuel cells biofixation. The results of digestate pretreatment and dilution, inoculum: digestate ratio and CO_2 supply system were reported. Each test was controlling operating temperature and light intensity using a common green alga, *Chlorella vulgaris*. Different tests were performed for testing digestate dilution (from 1:20 to 1:70) and for defining microalgal inoculum concentration (9%, 20% and 32%). All trials were performed at laboratory scale. The optimal digestate pretreatment and microalgal inoculum concentration was used for achieving CO_2 biofixation in an experimental reactor (15 L). The best dilution for the digestate was found to be approximately 1:70 and inoculum percentage equal to 20%.

1. INTRODUCTION

The distribution of high energy efficiency systems fed by renewable fuels is crucial for the future. Solid Oxide Fuel Cells (SOFC) are highly efficient energy systems that can be fed by renewable fuels (Choudhury, Chandra, & Arora, 2013; Eveloy & Gebreegziabher, 2019; Hagen, Rasmussen, & Thydén, 2011; Kupecki et al., 2018; Andrea; Lanzini, Leone, & Asinari, 2009; Papurello, Menichini, & Lanzini, 2017; Saadabadi et al., 2019; Shiratori, Ijichi, Oshima, & Sasaki, 2010). Experimental results in this field, from laboratory scale to pilot plant systems are encouraging, considering the integration between the real networks and the intermittency of renewable sources (A. Lanzini, Ferrero, Papurello, & Santarelli, 2017; Papurello, Iafrate, Lanzini, & Santarelli, 2017; Papurello & Lanzini, 2017; Papurello, Lanzini, Leone, & Santarelli, 2016; Papurello, Lanzini, Tognana, Silvestri, & Santarelli, 2015; Papurello, Silvestri, Tomasi, Belcari, et al., 2016b; Papurello, Tomasi, Silvestri, Belcari, et al., 2016; Papurello, Tomasi, Silvestri, & Santarelli, 2016). The ability to remove and to fix carbon dioxide from SOFC exhausts is also a crucial theme to be addressed for future development. Such benefits are related to the high purity of these exhausts, which does not require strong methods of cleaning, to avoid the pollutants interaction with the biological route for the algae growth (Santarelli, Briesemeister, Gandiglio, Herrmann, Kuczynski, Kupecki, Lanzini, Llovell, Papurello, Spliethoff, Swiatkowski, Torres-Sanglas, et al., 2017). Among several methods, carbon dioxide can be removed or separated from a gas flow with adsorption or absorption methods, or using membranes or biological systems (Rasi, 2009). Among biological methods, carbon dioxide can be fixed into new biomass through algae production (Molino, Nanna, Ding, Bikson, & Braccio, 2013). A strongly connected benefit is the sink generation for the biogenic CO_2 removal, with the biomass circular production approach. Microalgae can grow 10-50 times faster than terrestrial plants because they can fix captured solar energy as high-energy-density lipids (Ho, Kondo, Hasunuma, & Chang, 2013). Also microalgae do not have roots, shoots, and leaves that represent energy sinks. During photosynthesis, CO_2 is converted into sugars, inter alia, with the use of energy derived from ATP (adenosine triphosphate 5) and with the participation of the Rubisco-ribulose bisphosphate carboxylase oxygenase enzyme in the Calvin Cycle (Packer, 2009). Efficiency

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of microalgal photosynthesis can range from 3% (*Spirulina* sp.) to 20% (*Chlorella* sp.). Moreover, because of their high concentration of cellular lipids, microalgae can be used as feedstock for biofuel production, such as biodiesel (Strange, Hastings, & Wales, 2010). The use of microalgae is seen as a reasonable and efficient way of biofixation of CO_2 (Benemann, 1997; Rosenberg, Mathias, Korth, Betenbaugh, & Oyler, 2011). Biological methods of CO_2 capture from gas exhausts are potentially useful and need to be evaluated. Few studies have treated the integration of microalgal production and SOFCs exhausts (Santarelli, Briesemeister, Gandiglio, Herrmann, Kuczynski, Kupecki, Lanzini, Llovell, Papurello, Spliethoff, Swiatkowski, Torres-sanglas, et al., 2017). The SOFCexhaust could employed to sustain microalgae growth due CO_2 content expected in the range between (29-38.7%vv) (Santarelli, Briesemeister, Gandiglio, Herrmann, Kuczynski, Kupecki, Lanzini, Llovell, Papurello, Spliethoff, Swiatkowski, Torres-Sanglas, et al., 2017). The SOFC exhausts could better than other exhaust due the biogas was cleaned by the some important contaminants before feeding the stack (Papurello, Schuhfried, et al., 2015; Papurello, Silvestri, Tomasi, Belcari, et al., 2016a; Papurello, Tognana, et al., 2015; Papurello, Tomasi, & Silvestri, 2018; Papurello, Tomasi, Silvestri, Belcari, et al., 2016). Considering that the SOFC exhaust composition is now under study, the aim of this work is to perform at lab scale a system suitable to capture CO_2, simulating the SOFC exhaust. The exhausts originated from a SOFC system fed by biogas and the digestate were produced by the anaerobic digestion of organic fraction of municipal solid waste (OFMSW) in dry mesophilic anaerobic reactor [20]. The experimental goal was to investigate the main parameters affecting microalgal production using diluted digestate and simulated SOFC exhaust. The parameters considered were the best digestate dilution to avoid growth inhibition phenomena, the microalgal inoculum concentration, and the effect of CO_2 supply. These preliminary results on the experimental condition needed to obtain a good CO_2 fixation rate that will used in the future experimental tests with pilot scale reactors and with CO_2 supplied from SOFC exhaust – to – air, in order to use digestate as growing substrate for new biomass, algae, using CO_2 as a circular approach inside the biomass considered chain.

1.1 Nomenclature

A	is the area of the chamber (mm^2)
a	is the area of the field of view of the microscope
ATP	adenosine triphosphate
B1	dilution 1:20, 20% inoculum
B2	dilution 1:50, 20% inoculum
B3	dilution 1:70, 20% inoculum
B4	dilution 1:70, 9% inoculum
B5	dilution 1:70, 20% inoculum
B6	dilution 1:70, 32% inoculum
N°	is the algal counted on the slide
Ni	is the number of the squares counted
R1	dilution 1:20, 0% inoculum, CO_2 addition
SOFC	Solid Oxide Fuel Cells
V	was the volume considered

2. MATERIALS AND METHODS

The digestate from municipal solid waste was obtained at the end of a biogas production process at FEM (San Michele a/A, Trento). The process is described in detail elsewhere (Papurello et al., 2012). The digestate was collected after 30 days of mesophilic dry anaerobic digestion. The digestate was characterized about pH, TS content driyng at 105°C and VS content by burining the dry matter obtained at 550°C. The digestate was sieved at 2 mm and diluted (v/v) at different percentage in order to define the best dilution and to avoid turbidity inhibition of microalgae growth. The digestate was than sterilized in an autoclave at 121°C for 20 min.

The inoculum was a common green alga; *Chlorella* vulgaris (strain *Chlorella* vulgaris K-1801 SCAAP-DK). The microalga was initially grown in algal broth culture medium at 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 gentile centrifugation and washed twice with distilled water before inoculation to insure no nutrient carryover.

CO_2 was provided simulating SOFC exhausts completely dried after a condenser section and with no gas pollutants. A representative SOFC generator exhausts was studied elsewhere (Santarelli, Briesemeister, Gandiglio, Herrmann, Kuczynski, Kupecki, Lanzini, Llovell, Papurello, Spliethoff, Swiatkowski, Torres-sanglas, et al., 2017) and now is under investigation for another research work. Carbon dioxide along with liquid water are components that are easily removed. CO_2 flow was provided with a Mass Flow Controller (Bronkhorst, The Netherlands) using a gas cylinder (Siad, Italy).

The tests were accomplished with 250 mL Erlenmeyer flasks. The laboratory scale system was built with a 15-liter volume reactor for verifying the algal growth with CO_2 supply 2 mL min^{-1}.

Algal growth was tested under conditions listed in Table 1 for defining the digestate dilution (Tests B1-B3), algal inoculum (Tests B4-B6) and the CO_2 supply (R1).

Digestate dilution is the ratio between 1 moles of digestate and, 20 to 70 moles of water. Microalgal growth was determined by cell concentration. Every three days 0.5 mL of inoculated digestate was sampled, diluted with 3 mL of distilled water and fixed with Lugol's solution (SigmaAldrich, Germany). Algal cells were counted with a Fuchs-Rosenthal hemocytometer at 200 X magnification and measured with a calibrated ocular micrometer to determine biovolume. At least 400 cells were counted for each sample and cells were separated into small (6 mm), medium (10 mm) and large (15 mm) cells, measuring 25 cells per size class. Biovolume was calculated according to equation (1) and expressed in (mm^3 mL^{-1}). All samples were replicated three times.

$$N°/mL = Ni \times A/a \times 1/Ni \times 1/V \quad (1)$$

Where:
N° is the algal counted on the slide;
A is the area of the chamber (mm^2);

a is the area of the field of view of the microscope;
Ni is the number of the squares counted and V was the volume considered.

3. RESULTS AND DISCUSSION

3.1 Digestate dilution and microalgal inoculum tests

Algae growth using digestate (from the anaerobic digestion batch) as the culture medium was analyzed. Preliminarily it was defined the dilution effect, the percent algae inoculum added to the starting batch

The raw digestate have 29.80% of TS, 17.56% of VS and pH 8.54. The digestate dilution effect on TS content was reported in Table 2.

The batch results and the trial description are reported in the Figure 1. The best dilution for the digestate was found to be at 1:70 (B3), giving a cell biovolume of around 11.24 mm^3 mL^{-1} at the end of the trial (28 days), 79% and 81% higher than the dilution of 1:50 (B2) and 1.20 (B1), respectively. Diluting the digestate 30 times instead of five times enhanced the growth of *C. vulgaris*, *C. sorokiniana* and *Scenedesmus spp.* strains (Zuliani et al., 2016). The highest digestate concentration (dilution 1:20 and 1:50 of raw digestate) gave the lowest algal growth (Figure 1). 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. From our results, the TS concentration of digestate must be less than 4.5 g L^{-1} to avoid turbidity inhibition of microalgal growth and guarantees a sufficient light penetration. Higher values of TS content probably prevent the microalgal growth.

The test (B5) (+20%) had the highest algae growth with a total production of 25.57 (mm^3 mL^{-1} - Figure 2). The highest nominal inoculum condition (B6) and the lowest (B4) inoculum condition had the lowest biovolume (9.43 and 19.66 (mm^3 mL^{-1}, respectively). At the end of the process, B6 showed an increasing trend lower than the nominal case of 20% (B5). As reported by Uggetti et al. (2014) microalgal growth was positively correlated with the initial inoculum and digestate concentration (Uggetti, Sialve, Latrille, & Steyer, 2014). Higher initial microalgal concentration produced more biomass. The inoculum size of our tests was about 0.78 mm^3 mL^{-1}, 1.18 mm^3 mL^{-1} and 1.85

TABLE 1: Experimental conditions: digestate dilution is the ratio between digestate and water; algal inoculum is the percentage of microalgae culture added to the digestate for the initial growth; CO_2 addition is the condition with or not CO_2 and the percentage of loading.

trial	digestate dilution	algal inoculum	CO_2 addition
B1	1:20	20%	no
B2	1:50	20%	no
B3	1:70	20%	no
B4	1:70	9%	no
B5	1:70	20%	no
B6	1:70	32%	no
R1	1:70	20%	350 ppm (v)

TABLE 2: Digestate dilution and TS concentration of the different tests.

trial	digestate dilution	TS content
B1	1:20	14.90 g L-1
B2	1:50	5.96 g L-1
B3-B6	1:70	4.25 g L-1

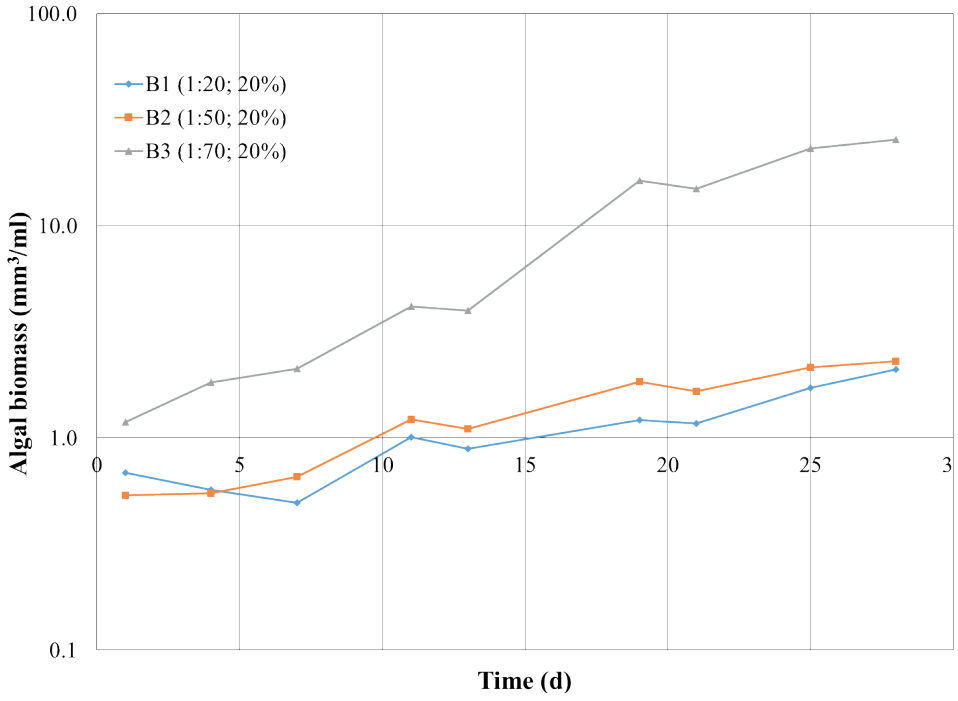

FIGURE 1: Digestate dilution effect B1=1:20, B2=1:50, B3=1:70.

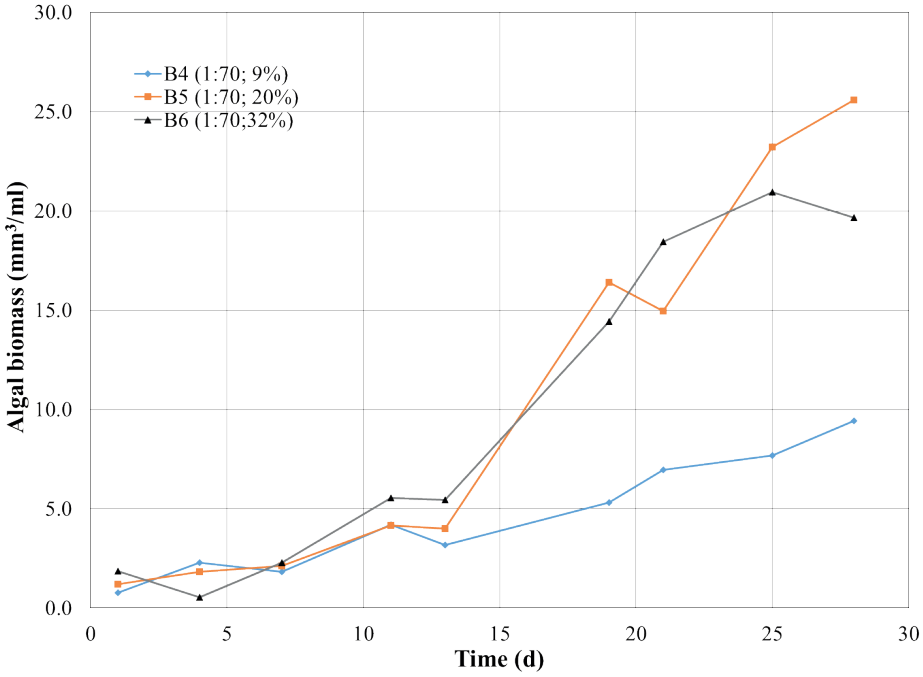

FIGURE 2: Inoculum effect B4=+9%, B5=+20%, B6=+32%.

mm^3 mL^{-1} for 9%, 20% and 32% of inoculum size respectively, that correspond at 4.48 $10E^6$ cell mL^{-1}, 1.10 $10E^7$ cell mL^{-1} and 1.87 $10E^7$ cell mL^{-1}. In agreement with Lau et al. (1995), (Lau, Tam, & Wong, 1995) the super concentrated culture of *Chlorella* vulgaris (with an initial inoculum of 1E107 cells mL^{-1}) did not exhibit any self-shading limitation of growth and nutrient removal.

3.2 Carbon dioxide supply tests

Figure 3 shows algal growth for R1, enriched with CO_2 (350 ppm (v)) continuously for two months and the digestate was diluted according to the best results identified in the previously trials. The microalgal biovolume was similar to laboratory tests (23.31 (mm^3 mL^{-1}), comparable with the results of B5 at the same digestate concentration and inoculum dosage (25.57(mm^3 mL^{-1}). The algal growth was different comparing to the preliminary tests on Erlenmeyer flask, highlighting the effect on mixing system and acclimation of the microalgae to anaerobic medium. The microalgal inoculum was prepared using nitrate as main nitrogen source, while in the diluted digestate used as growth medium the ammonia is prevalent. Previous work conducted in similar condition has been demonstrated that the ammonia was preferred N substrate [24]. Our results confirm this effect, but highlighted that when the medium volume increased a higher lag-phase could occur. This effect suggests that a short acclimation time at aerobic condition of anaerobic diluted digestate with ambient air, could decrease the very long lag phase. Another reason regarded to the mixing system adopted. Increasing the bubbling CO_2 mixed to ambient air up to 0.2 vvm will guarantee a better medium blending promoting a better light penetration in agreement with Ryu et al., 2009 (Ryu, Oh, & Kim, 2009).

pH was measured during the process to understand why algal production did not increase substantially. R1 showed a low pH value in the lag phase of microalgal growth compared to B5 (6.2 vs 9.2). The starting pH of R1 reactor was 8.5 and it was acidified following CO_2 enrichment. During the logarithmic growth of microalgae, pH decreased to 7.8 following the CO_2 uptake in microalgal cells.

Our results were slightly lower than other studies of microalgae cultivated in liquid digestate (Franchino, Comino, Bona, & Riggio, 2013; Uggetti et al., 2014; Xia & Murphy, 2016). The main inhibition phenomena of algal growth were due to the particulate matter content and turbidity, as well as to the color of the starting digestate, despite high dilutions.

4. CONCLUSIONS

The best dilution for the digestate was found to be approximately 1:70 and inoculum percentage equal to 20%. Algal growth coupled to CO_2 fixation by algae was verified experimentally, using the best conditions identified in the laboratory tests. The present work provided preliminary information about algal growth with digestate from dry anaerobic digestion of OFMSW and CO_2 from SOFC exhausts. These results will allow performing specific tests with experimental photobioreactors to verify algal productivity, ammonia and phosphate removal efficiency from digestate, CO_2 fixation rate and CO_2 fixation efficiency. This work is currently underway.

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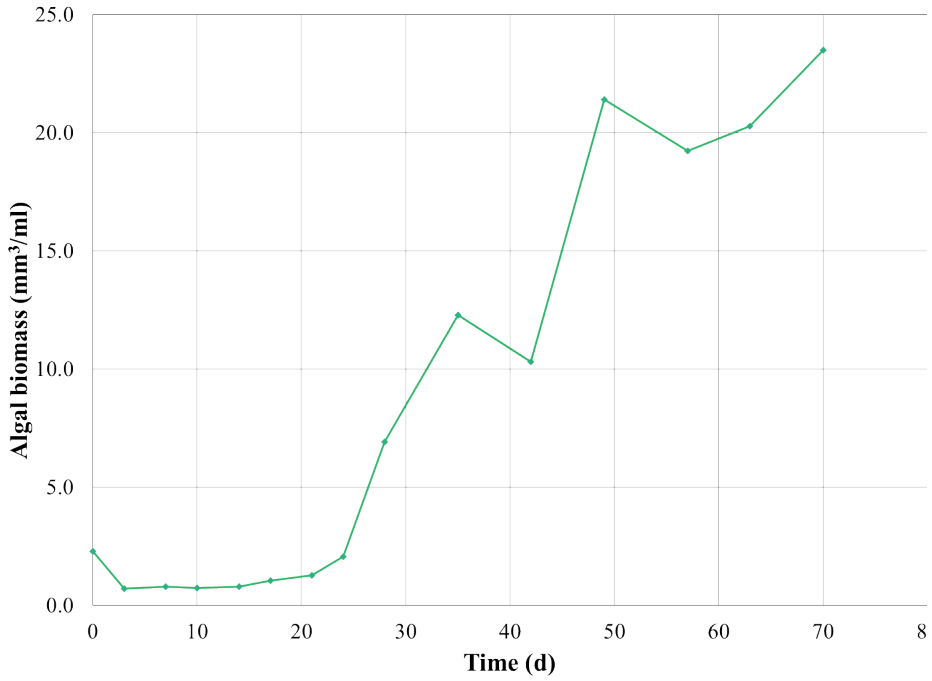

FIGURE 3: Experimental photobioreactor with digestate at 1:70 dilution and CO_2 350 ppm(v) continuously feeding.

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