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Anaerobic digestion of orange peel in a semi-continuous pilot plant: an environmentally sound way of citrus waste management in agro-ecosystems

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Abstract

The management of residues of citrus processing involves economic and environmental problems. In particular, the uncontrolled disposal of citrus processing waste near production sites can have heavy impacts on air, soil, surface water bodies and groundwater. Anaerobic digestion has been proposed as a viable alternative for citrus waste valorisation, if some problems, linked to the biochemical processes, are overcome.

Although many experimental tests have studied the inhibitory effects of the high essential oil content of orange peel on biomethanisation processes, fewer experiences have been carried out in continuous or semi-continuous pilot digesters, more similar to the full-scale biogas plants, using real orange peel.

This study has evaluated the methane production through anaerobic digestion of industrial orange peel using a pilot plant (84 litres) with semi-continuous feeding at increasing Organic Loading Rates (OLR) and essential oil (EO) supply rates (EOsr) until the complete process inhibition.

Under mesophilic conditions, the highest daily specific methane yield was achieved at OLR of 1.0 g TVS L^{-1} d^{-1} and EOsr of 47.6 mg L^{-1} d^{-1}. Partial inhibition of the anaerobic digestion was detected at OLR and EOsr of 1.98 g TVS L^{-1} d^{-1} and 88.1 mg L^{-1} d^{-1}, respectively and the process irreversibly stopped when OLR and EOsr reached 2.5 g TVS L^{-1} d^{-1} and 111.2 mg L^{-1} d^{-1}, respectively. Under thermophilic conditions, the cumulative methane production (0.12 L g TVS^{-1}) was about 25% of that under mesophilic conditions (0.46 L g TVS^{-1}). The thermophilic digestion was completely inhibited at lower OLR (1.98 g TVS L^{-1} d^{-1}) and EOsr (88.1 mg L^{-1} d^{-1}) compared to mesophilic conditions.

This study confirmed the suitability of anaerobic digestion of orange peel for biomethane production (provided that the right management of the process is set), in view of an environmentally sound way of agricultural residues management in agro-ecosystems.

Keywords: *essential oil; methane yield; biogas; process inhibition; organic loading rates; limonene.*

1. Introduction

Cultivation of citrus fruits (primarily oranges) plays an important role in the agricultural sector of many countries (e.g., United States, Brazil, Mexico, China and, in the Mediterranean basin, Spain, Greece and Italy) (Martin et al., 2010; Koppar and Pullammanappalil, 2013). A share of about 30-50% of produced fruits is consumed fresh, while the remaining part is sent to industrial transformation for juice, marmalade, pectin and other foods production. Beside citrus wastewater, the industrial process produces large amounts of solid residues (peel, seeds and membranes) (Wilkins et al., 2007), which

represent over 50-60% of the processed fruits (Martìn et al., 2010). However, the management of citrus residues involves economic and environmental problems, due to the high amount produced (with possible air, water and soil pollution), the high processing cost and/or the low value of the final material recovered (Calabrò et al., 2016). In fact, in general, use as cattle feed is the traditional and most practised option for citrus residues disposal, but some environmental and economic concerns - such as percolation of pollutants and odours emission and insufficient request of cattle feed near the transformation industry - limit this destination.

In some contexts, there is no satisfactory mean of disposal other than dumping on land adjacent to the production sites (Martìn Santos et al., 2010; Pourbafrani et al., 2010), when more efficient and sustainable solutions lack. The dumping of citrus processing waste near production sites, as for other biodegradable waste, can have heavy impacts on air, soil, surface water bodies and groundwater. The uncontrolled rotting can lead to the emission of odours (Khoo et al., 2010) and greenhouse gases (Baur et al., 2015); these latter are produced even if citrus waste is disposed of in controlled landfills (Calabrò et al., 2015). Moreover the dumping on soil causes the release of a leachate rich in organic substances with adverse impacts not only on soil itself, but also on fresh and groundwater (Arukwe et al., 2012).

In the recent decades the use of orange peel (OP) as substrate for bioenergy production (namely bioethanol by alcoholic fermentation and biomethane by anaerobic digestion) (Ruiz and Flotats, 2014) has been suggested as a viable alternative for its management. This option not only reduces the environmental drawbacks linked to pollution from poor waste-management practices, but also allows the increase of citrus processing company profits by the recovered energy sale (with or without subsidies) (Zema, 2017). Theoretically, the high organic content of OP mainly consists of various soluble and insoluble carbohydrates and this makes this substrate suitable for biogas or ethanol production (Pourbafrani et al., 2010; Koppar and Pullammanappallil, 2013). In general, from the energy yield perspective, ethanol production as OP valorisation option is not as efficient as methane

production: the biochemical methane potential (BMP) of citrus peel is 1.5 - 2 times higher than the values obtained with bioethanol; in addition, by using co-digestion strategies, other by-products could be co-treated with the orange waste, thereby contributing to integral waste management within the producing area (Ruiz and Flotats, 2014). Thus, anaerobic digestion is the most promising process for energy conversion of this residue (Calabrò et al., 2016).

The main obstacle for OP use as a substrate for biogas production is its high content of essential oils (EO) (up to 0.4-0.5% w/w) (Kimball, 1999; Martin et al., 2010). This compound, mainly composed of D-limonene (86-96%) (Ruiz and Flotats, 2014), is highly toxic for microorganisms and thus EO presence decreases the biogas yield of OP anaerobic digestion (Mizuki et al., 1990; Pourbafrani et al., 2010; Ruiz and Flotats, 2014). Various processes have been developed to increase the energy efficiency of OP anaerobic digestion, such as several pre-treatments (e.g. size reduction, steam distillation, hexane extraction, addition of enzymes, steam explosion and combination of steam explosion and dilute acid hydrolysis) to remove D-limonene before the substrate is digested (Koppar and Pullammanappallil, 2013). However, these pre-treatments are in several cases expensive and thus unsustainable from an economic point of view. Dilution of EO present in OP by co-digestion with other feedstocks is another option.

Scientific literature reports many experimental tests of OP anaerobic digestion, but the experiments made on citrus co-digestion with other substrates are few (Mandal and Mandal, 1997; Martín et al., 2013). The important review of Ruiz and Flotats (2014) reports measurements of biogas and methane yields and the effects of EO concentration on citrus peel biomethanisation, as performed by several Authors; in summary, a wide range of inhibition limits for D-limonene are reported for anaerobic digestion: 24 mg L^{-1} d^{-1} (Srilatha et al., 1995); 34 mg L^{-1} d^{-1} (Forgács et al., 2011); 65 µL L^{-1} d^{-1} (Mizuki et al., 1990); 75-95 mg L^{-1} d^{-1} (Lane, 1984); 192 mg L^{-1} d^{-1} (Akao et al., 1992). The same authors found an inhibitory effect of EO at a concentration of about 200 mg L^{-1} during BMP

experiments (Ruiz and Flotats, 2016). With regards to the effects of OLR on methane production, under mesophilic conditions, Lane (1980), Mizuki et al. (1990) and Srilatha et al. (1995) reported an inhibition limit between 2.0 g L^{-1} d^{-1} of Total Solids (TS) - corresponding to 1.9 g L^{-1} d^{-1} of Total Volatile Solids (TVS) - and 2.5 g L^{-1} d^{-1} of TVS; under thermophilic conditions, according to Kaparaju and Rintala (2006) the OLR that makes the anaerobic process unstable is 5.6 g TVS L^{-1} d^{-1}.

However, beside the evident dispersion of data due to the different conditions adopted for the processes (e.g., the different inocula), the majority of these experiments have been carried out through laboratory batch tests and often by adding externally D-limonene to the substrate or removing EO before digestion. For instance, Calabrò et al. (2016) evaluated the effect of increasing the concentration of orange essential oil (up to 2 g L^{-1}), reporting methane yields up to 0.37 NL g TVS^{-1} in mesophilic and 0.30 NL g TVS^{-1} in thermophilic conditions; moreover, the same Authors observed that D-limonene is quantitatively transformed in *p*-cymene, but that other removal mechanisms could be present, as, for example, volatilization. With regards to specifically EO removal before digestion, many treatments, such as distillation, aeration, solid state fermentation, biological removal, steam explosion, solvent extraction, were investigated, in order to evaluate the influence of EO reduction on anaerobic digestion performance (Ruiz and Flotats, 2014): for example, under mesophilic conditions, Lane (1984) found that an OLR up to 3.5 g TS L^{-1} d^{-1} can be adopted. Considering that TVS are about 95% of TS (Ruiz and Flotats, 2014; Martin et al., 2010), this OLR corresponds to about 3.3 g TVS L^{-1} d^{-1}.

However, very few experimental tests of OP anaerobic digestion have been carried out in continuous or semi-continuous pilot digesters, which are (more similar to the full-scale biogas plants), using real OP. To fill this gap, this study aims to evaluate the methane production through anaerobic digestion of industrial orange peel using a pilot plant (84 litres) with semi-continuous feeding. More specifically, the specific methane yields have been measured under mesophilic and thermophilic conditions at increasing OLR and EO concentrations until complete process inhibition; the limits of OLRs and EO

supply of our study have been compared with literature data.

2. Materials and methods

2.1. Test description

The semi-continuous tests were carried out in a pilot anaerobic digester with a reactor volume of 84 litres for 43 days (Figure 1). The digester was very similar to the prototype described in the work by Bernardi et al. (2017).

The tests were carried out under mesophilic (42 °C) and thermophilic (51 °C) temperatures (henceforth indicated as "M" and "T"), respectively. The inoculum was a raw digestate taken from a commercial plant, fed mainly with manure and other agro-industrial residues (i.e. cheese whey, discarded fruit and vegetables). Twice a week, industrial ensiled OP, grinded with a commercial blender (Silvercrest 250 W) to facilitate digester feeding and diluted with conventional water at 5% TS (w/w), was added to the digester. Since OP is poor in nitrogen (Serrano et al., 2014), dried poultry manure (PM) was added (about 0.3 g TVS $L_{digester}^{-1}$ d^{-1}) from the 5th to the 7th and from the 11th to 17th day of operation, in order to balance the C/N ratio of the blend (Table 1).

For both tests the organic loading rate, initially set to 0.64 g TVS L^{-1} d^{-1}, was gradually increased up to 2.0 (test T) and 2.5 (test M) g TVS L^{-1} d^{-1}. The feeding diagram is reported in Figure 2. Since the EO concentration in the digester blend increases with OLR, this allowed the evaluation of the biogas/methane yield production also at increasing EO supply rates (henceforth "EOsr").

Figure 1

Figure 2

2.2. Substrate and inoculum characterization

The main physico-chemical parameters of raw substrates used for the tests (digestate, OP and PM) were determined before the tests (Table 1), measuring in triplicate (APHA et al., 2005):

- TS, by oven drying the wet biomass until weight stabilisation;
- TVS, by calcination at 550 °C of the dry matter;
- pH by portable pH-meter (XS Instruments);
- EO concentration (Scott and Velhuis, 1966).

Throughout the experiment, biogas production and chemical composition were recorded. Since the digester measured automatically and continuously the biogas pressure, the volume of biogas produced was estimated from these data, applying the real gas equation. The chemical composition (CH_4, CO_2, O_2, H_2S) of the produced biogas was daily measured by a portable device with suitable probes (MRU Airfair Optima 7 Biogas) and the same was made twice a week for pH on samples of the digestate taken from the reactor. The methane yields measured were referred to the TVS supplied daily with substrates into the digester; this production will be indicated henceforth as daily specific methane yield. Finally, the Hydraulic Retention Time (HRT) was calculated as the ratio between the digester volume and the volume of diluted OP supplied daily.

Table 1

2.3. Statistical analysis

The non-parametric Kruskal-Wallis test was used to test whether the values of the daily specific methane yields achieved between two consecutive substrate feeding operations into the digester were significantly different at $p < 0.05$. The non-parametric Mann-Whitney test allowed to control whether the daily specific methane yields measured were significantly different at $p < 0.05$ between mesophilic and thermophilic temperatures. The statistical analysis was performed using XLSTAT release 17.

3. Results

3.1. Mesophilic test

In the mesophilic test the methane volume appreciably increased until the 32th day of operation, then the production declined fast (Figure 3a). In the first 13 days EOsr was of 28.6 mg L^{-1} d^{-1}, OLR was equal to 0.64, when OP only was fed, and equal to 1.00 g TVS L^{-1} d^{-1} when PM was also added (Figure 2). In this period the daily specific methane yield was in the range 0.27-0.49 L g TVS^{-1} d^{-1} (Table 2), while methane content of biogas was between 56.0 and 59.0% (Figure 3a).

On the 14th day EOsr was increased to 47.6 mg L^{-1} d^{-1} and OLR to 1.07 g TVS L^{-1} d^{-1} (OP only) corresponding to a total OLR of 1.38 g TVS L^{-1} d^{-1} when PM was added on day 14 (Figure 2). The maximum daily specific methane yield got a maximum of 1.22 L g TVS^{-1} d^{-1} and the mean value was recorded between the 18th and the 21st day, which is significantly different, $p < 0.05$, from the methane yields previously measured (Table 2); methane content in the biogas was always higher than 60% with a peak of 68% on the 21th day (Figure 3a).

PM addition was stopped on the 18th day and in the remaining part of the experiment the reactor was fed only with OP, since the concentration of H_2S in biogas was 4177 ppm; values over 4000 ppm are excessive according to Chen et al. (2008). From the 22nd to the 34th day, the OLR and EOsr were set at 1.98 g TVS L^{-1} d^{-1} and 88.1 mg L^{-1} d^{-1}, respectively (Figure 2). As a process response, the mean methane yield progressively decreased, but not significantly ($p < 0.05$). The specific methane yield in the period 22nd-34th day was equal to 0.67 L g TVS^{-1} d^{-1}, very similar (at $p < 0.05$) to the mean value recorded from the 14th to the 17th day (0.68 L g TVS^{-1} d^{-1}) and the process did not show inhibition (Table 2). In this period, the methane content of the biogas was on average equal to 66.0%, with an outlier of 47.0% measured on the 26th day (Figure 3a).

From the 35th to 39th day, OLR and EOsr were increased to 2.5 g TVS L^{-1} d^{-1} and 111.2 mg L^{-1} d^{-1}, respectively (Figure 2); under these loads daily specific methane yield and methane content in biogas decreased under 0.04 L g TVS^{-1} d^{-1} and 33.4%, respectively (Figure 3a and Table 2), significantly different ($p < 0.05$) from the methane yields of the previous periods. This reduction was probably due to a process inhibition because of EO accumulation. By decreasing OLR and EOsr from the 40th day until the test end (43rd day) to the values set for the antecedent period, the process did not restart, as shown by the negligible methane yield.

At the end of the mesophilic test the cumulative methane yield was 30.5 L $L_{digester}^{-1}$, corresponding to a specific total production of 0.46 L g TVS^{-1}; 98% of the production was in the first 34 days.

In our test, pH was in the optimal range for a balanced anaerobic process (6.5-8.5) (Wikandari et al., 2015; Gonzàles-Gonzàles and Cuadros, 2015; Martinez-Garcia et al., 2009) until the 34th day of operation. Then a fast decrease was observed and the final pH was 3.55 (Table 2). Lane (1980) reports a failure of mesophilic anaerobic digestion of orange peel at pH lower than 6.4.

During the whole test hydraulic retention time was in the range normally adopted in full scale anaerobic digestion plants (15-30 days) or higher (Figure 3).

3.2. Thermophilic test

During the thermophilic test methane yield was appreciable (over 0.10 L g TVS^{-1} d^{-1}) only in the first 25 days (Table 2). In the first 13 days, at OLR (OP only) of 0.64 (1.00 g TVS L^{-1} d^{-1} considering also PM) and EOsr of 28.6 mg L^{-1} d^{-1} (Figure 2), the daily specific methane yield and content in the biogas steady and significantly (at $p < 0.05$) increased from 0.11 L g TVS^{-1} d^{-1} and 19.8% (days 1-4) up to a maximum value of 0.40 L g TVS^{-1} d^{-1} and 48.7% (8th-10th day), respectively (Table 2 and Figure 3b). Then, the mean daily specific methane yield decreased to 0.28 L g TVS^{-1} d^{-1} (days 11-13, but not significantly at $p < 0.05$) (Table 2), but average methane content increased to 53.4% (Figure 3b).

From the 14th to 21st day, OLR supplied with OP was increased to 1.07 g TVS L^{-1} d^{-1} (and to 1.38 g TVS L^{-1} d^{-1} when PM was added) and EOsr was set at 47.6 mg L^{-1} d^{-1} (Figure 2). At these loads, the maximum specific methane yield was recorded between the 18th and 21st day (0.33 L g TVS^{-1} d^{-1}), which is not significantly different from the previous period at $p < 0.05$ (Table 2), but the average methane content of the biogas in this period was quite low (37.6%) (Figure 3b). Since the H_2S concentration in the biogas was higher than 16000 ppm, PM supply was stopped at the 18th day.

From the 22nd day (that is, when the EOsr was increased to 88.1 mg L^{-1} d^{-1} and the OLR was set at 1.98 g TVS L^{-1} d^{-1}) (Figure 2), the methane content of the biogas suddenly dropped (under 30% on the 25th day, Figure 3b) and the daily specific methane yield significantly (at $p < 0.05$) decreased to 0.05 L g TVS^{-1} d^{-1} (Table 2), which indicated a severe inhibition of the process. On the 31st day the methane content in the biogas was negligible and the methane yield practically null (Figure 3b).

At the end of the thermophilic test the cumulative methane yield was 7.68 L $L_{digester}^{-1}$, corresponding to a specific total production of 0.12 L g TVS^{-1}.

As for the mesophilic test, pH was in the optimal range (between 7.4 and 8.1) throughout the first 21 days; subsequently, pH dropped down to the final value of 5.6 (Table 2). Also during the thermophilic test HRT was in the range normally adopted in full scale anaerobic digestion plants (15-30 days) or higher (Figure 3).

Figure 3

Table 2

4. Discussions

During the mesophilic test methane production was substantial after 10-15 days and until the 34th day (Table 2). The anaerobic digestion process started slowly with a moderate methane production rate for about 10-13 days at lower OLR and EOsr (< 1.00 g TVS L^{-1} d^{-1} and 28.6 mg L^{-1} d^{-1}, respectively). These methane production rates increased at higher organic and EO loads, up to 1.38 g TVS L^{-1} d^{-1} and 47.6 mg L^{-1} d^{-1}, respectively, between the 14th and the 21st day. A reduction of the process yield was recorded in the subsequent 13 days, at OLR of 1.98 g TVS L^{-1} d^{-1} and EOsr of 88.1 mg L^{-1} d^{-1}. Therefore, an EO supply rate of 88.1 mg L^{-1} d^{-1} does not seem to severely inhibit the anaerobic digestion, but it slows down the biochemical process, while the process is more stable at an EO load close to 47.6 mg L^{-1} d^{-1}. When OLR and EOsr were set at values of 2.5 g TVS L^{-1} d^{-1} and 111.2 mg L^{-1} d^{-1} (after 34 days), respectively, the methane production rate and content in biogas progressively and irreversibly stopped, presumably due to an accumulation of EO and of its metabolites (Calabrò et al., 2016).

At the end of the mesophilic test the cumulative methane yield was 0.46 L g TVS^{-1}. This specific production complies with the results of mesophilic tests carried out by several Authors. In general, the methane yields reported in literature for semi-continuous anaerobic digesters fed with orange peel under mesophilic conditions fall in a wide range, i.e. from 0.11 to 0.50 L g TVS^{-1} (Lane, 1984; Srilatha et al., 1995; Chanakya et al., 2009). Presumably, the low methane yield detected by Chanakya et al. (2009) is due to the low inoculum concentration into the digester blend or to a too short adaptation period of microbial population (Ruiz and Flotats, 2014).

According to our experimental results, an OLR up to 1.98 g TVS L^{-1} d^{-1} seems to be acceptable, because at this load only a very slight methane reduction is achieved compared to lower OLRs; the process was inhibited at OLR of 2.5 g TVS L^{-1} d^{-1}. These results basically confirm the OLR limits reported in the literature for the digesters fed with citrus peel. Mizuki et al. (1990) found that anaerobic digestion of OP (at 37 °C) was stable at OLR of 1 g L^{-1} d^{-1} of Total Solils (TS) and failed at 2 g TS L^{-1} d^{-1} (about 1.9 g TVS L^{-1} d^{-1}); the same value for OLR limit was detected by Lane (1980), while Srilatha et al. (1995) found that anaerobic digestion was inhibited at OLR of 2.5 g TVS L^{-1} d^{-1}. These latter Authors estimated this OLR limit, carrying out experimental tests at 30 °C of anaerobic digestion of pre-treated OP by solid-state fermentation (82.4% of D-limonene removal). Concerning the EO inhibiting effect on anaerobic digestion of citrus residues, from our mesophilic test it emerges a full stability up to 47.6 mg L^{-1} d^{-1}, a decrease in the average methane production at EOsr of 88.1 mg L^{-1} d^{-1} and a strong process inhibition at 111.2 mg L^{-1} d^{-1} (Table 3). Literature data show methane production inhibition at D-limonene (90-95% of EO content) concentrations between 24 mg L^{-1} d^{-1} (Srilatha et al., 1995) up to even 192 mg L^{-1} d^{-1} (Akao et al., 1992).

PM addition may have helped to stabilize the process, since it is rich in nitrogen compounds. Some Authors highlighted that many strains of denitrifying bacteria (*Alcaligenes defragrans*) are able to

break the D-limonene structure in presence of nitrate (Foss et al., 1998) and to convert iso-limonene into iso-terpinolene (Heyen and Harder, 1998), easy to be digested by bacterial enzymes.

Table 3

In the literature the effects of pre-treatments to reduce the EO inhibiting effects on anaerobic digestion were also investigated: Lane (1984) found an OLR limit of 3.5 g TS L^{-1} d^{-1} (about 3.3 g TVS L^{-1} d^{-1}); for anaerobic digestion of comminuted peel diluted, with water at 10% of TS (w/v), after EO extraction by distillation and of 3 g TS L^{-1} d^{-1} (about 2.9 g TVS L^{-1} d^{-1}) after EO recovery in a juice extraction factory; Mizuki et al. (1990) found that no inhibition was observed after removal of essential oil by steam distillation or aeration with an OLR of 2.0 g TS L^{-1} d^{-1} (about 1.9 g TVS L^{-1} d^{-1}). Srilatha et al. (1995), studying the anaerobic digestion of pre-treated orange peel by solid-state fermentation (82.4% of D-limonene removal, down to 24 mg L^{-1} d^{-1}), found an OLR limit of 2.5 g_{VS} L^{-1} d^{-1}.

Under thermophilic conditions the methanogenic activity was appreciable only in the first 21 days at OLR between 0.64 and 1.38 g TVS L^{-1} d^{-1} and EOsr between 28.6 and 47.6 mg L^{-1} d^{-1}. Afterwards, the process seemed to be subject to a sudden inhibition at EOsr of 88.1 mg L^{-1} d^{-1} and OLR of 1.98 g TVS L^{-1} d^{-1}. Finally, from the 31st day the anaerobic process stopped almost completely (OLR of 1.98 g TVS L^{-1} d^{-1}).

The average methane yield in our test before process inhibition (about 0.30 L g TVS^{-1} d^{-1}) is very close to the value detected in literature for semi-continuous anaerobic digesters under thermophilic conditions. These methane yields are in the range of 0.30 - 0.60 L g TVS^{-1} (Kaparaju e Rintala, 2006; Martin et al., 2010; Siles et al., 2016). In more detail, Kaparaju and Rintala (2006) reported methane yields of 0.49 L g TVS^{-1} in batch tests and 0.60 L g TVS^{-1} in semi-continuous digesters (OLR of 2.8 g TVS L^{-1} d^{-1} after 26 days); operating at OLR of 4.2 g TVS L^{-1} d^{-1}, after 40 days the methane yield was

about 0.5 L g TVS^{-1} and a further increase of OLR (5.6 g TVS L^{-1} d^{-1}) gave a complete block of methanogenesis, a drop in methane content and a pH reduction. Martìn et al. (2010), after removing D-limonene by steam distillation under 2 mg L^{-1}, observed higher methane yields (0.33 L g TVS^{-1}) under thermophilic conditions compared to mesophilic temperatures (0.23 L g TVS^{-1}) and found OLRs between 1.20-3.67 g_{COD} L^{-1} d^{-1} as optimal values. Forgàcs et al. (2011) reported methane yields of about 0.55 L g TVS^{-1} d^{-1} for citrus waste treated with steam explosion and in co-digestion with the organic fraction of municipal solid waste (30/70%, respectively) with an OLR of 3 g TVS L^{-1} d^{-1}. Siles et al. (2016), after reducing the concentration of D-limonene by steam distillation (with removal efficiency around 70% after one hour), achieved a methane yield of 0.28 L g_{COD}^{-1} at a maximum OLR of 3.67 g_{COD} L^{-1} d^{-1} without acidification and process inhibition.

With regards to the process tolerance to OLR and EO, from our thermophilic test no evident process inhibition was detected until an OLR of 1.4 g TVS L^{-1} d^{-1} and EOsr of 47.6 mg L^{-1} d^{-1} (Table 3). The negative effect of thermophilic conditions could be linked to the higher mobility of EO at this temperature or to the lower tolerance of thermophilic bacterial strains (Ruiz and Flotats, 2014). Wikandari et al. (2014) found an increase of methane yield up to OLR of 1.5-2.0 g TVS L^{-1} d^{-1} and D-limonene concentration of 60 mg L^{-1} d^{-1}; at OLR of 2.5 and 3.0 g TVS L^{-1} d^{-1} and D-limonene concentration around 75 - 90 mg L^{-1} d^{-1}, the methane production quickly decreased. Sudden decreases of pH at OLR of 1.91 g TVS L^{-1} d^{-1}, similarly to what observed in this study at 1.98 g TVS L^{-1} d^{-1}, are reported by Martin et al. (2013). Kaparaju and Rintala (2006) found that an OLR of 5.6 g TVS L^{-1} d^{-1} made the anaerobic process unstable, suggesting a related value lower than 4.2 g TVS L^{-1} d^{-1} to avoid inhibitory effects; these Authors did not report inhibition limits due to EO presence. The results of the literature experiments carried out to reduce the EO inhibitory effects on thermophilic anaerobic digestion show that: after removing D-limonene by steam distillation under 2 mg L^{-1}, the optimal OLR was between 1.20–3.67 kg_{COD} m^{-3} d^{-1} (Martìn et al., 2010); by using steam distillation to reduce the

concentration of D-limonene, a maximum OLR of 3.67 kg_{COD} m^{-3} d^{-1} can be achieved without acidification and process inhibition (Siles et al., 2016); biological removal by fungi or recovery steam distillation and ethanol extraction of D-limonene resulted in concentration decreases of 22%, 44% and 100%, respectively (Ruiz and Flotats, 2016) and, as expected, the methane yields and rate of the treated orange peel increased significantly after the pre-treatments.

Overall, the comparison between our mesophilic and thermophilic tests indicate that the cumulative specific methane yield (0.46 L g TVS^{-1}) in mesophilic conditions is about 4-fold the value measured in thermophilic conditions (0.12 L g TVS^{-1}); the differences between the specific methane yields achieved under mesophilic and thermophilic conditions were always statistically significant (at $p < 0.05$).

5. Conclusions

The methane yields of a semi-continuous pilot anaerobic digester fed with OP at increasing OLR and EO concentrations were evaluated under mesophilic and thermophilic conditions. The experimental tests showed that under mesophilic conditions the highest daily specific methane yield was achieved at OLR of 1.0 g TVS L^{-1} d^{-1} and EOsr of 47.6 mg L^{-1}. At higher OLR and EOsr (1.98 g TVS L^{-1} d^{-1} and 88.1 mg L^{-1}, respectively) partial inhibition was detected. The anaerobic process irreversibly stopped when OLR and EOsr got 2.5 g TVS L^{-1} d^{-1} and 111.2 mg L^{-1}.

The methane yields measured under thermophilic conditions were noticeably lower: the cumulative methane production (0.12 L g TVS^{-1}) was about 25% of the corresponding yield achieved under mesophilic temperature (0.46 L g TVS^{-1}). Moreover, the thermophilic digestion was found to be more influenced by high OLR and EOsr compared to the mesophilic process, being the total inhibition limit lower (OLR of 1.98 g TVS^{-1} L^{-1} d^{-1} and EOsr of 88.1 g TVS^{-1} L^{-1} d^{-1}) compared to mesophilic conditions.

The results of our study highlighted that feeding operations of an anaerobic digester should be made with caution. In other words, the organic and EO loads must be properly dosed, keeping the daily rates well below the inhibition limits. As reported in Table 3, which summarises the OLR and EOsr limits detected in our experimental tests, the mesophilic digestion was totally inhibited at OLR of 2.5 g TVS L^{-1} d^{-1} and EOsr of 111.2 mg L^{-1} d^{-1}, while in thermophilic process total inhibition occurred at 1.98 g TVS L^{-1} d^{-1} of OLR and EOsr of 88.1 mg L^{-1} d^{-1}. However, while the mesophilic process was subject to a partial inhibition at OLR of 1.98 g TVS L^{-1} d^{-1} and EOsr of 88.1 mg L^{-1} d^{-1}, under thermophilic temperature the anaerobic digestion suddenly underwent to total inhibition.

When setting operational condition not only OLR and, consequently, EOsr must be considered. In fact, the high HRTs connected to the low OLRs adopted in the first phase of the experiments are not beneficial for the process since they favour the accumulation of EO in the reactor and thus the inhibition. For this reason a wise use of co-digestion would be beneficial. However it could not be feasible when managing peak OP production and therefore research is still needed in order to find technical solutions for increasing applicable OLR.

On the whole, the experimental tests confirmed the suitability of anaerobic digestion of OP for biomethane production, provided that the right management of the process is properly set. Further test are planned to confirm the findings of this research and to optimize operational parameters (e. g., HRT, OLR, EO).

The study contributes to consolidate in the Mediterranean agriculture a sustainable practice (i.e. the anaerobic digestion) able to convert a potentially pollutant organic waste (i.e. the orange peel) - that can produce severe impact on air, soil and water - into a valuable source of profit (coming from biomethane sale or self-exploitation) for farmers and agricultural businessmen, in view of an environmentally and economically sound way of agricultural residues management in the agro-ecosystems.

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TABLES

Table 2 - Main physico-chemical parameters of substrates (mean ± std. devs., n = 3 replicates) and inoculum used for the anaerobic digestion tests.

Parameter	**Substrates**		
	Inoculum	*OP*	*PM*
pH	8.21±0.01	3.59±0.03	6.82±0.01
TS (%)	4.95±0.02	10.0±0.04	93.8±0.25
TVS (%TS)	80.8±0.35	89.6±0.35	85.0±0.29
EO (%)	0.00±0.00	0.40±0.02	0.00±0.00
C/N	12.9±0.02	42.2±2.33	5.82±0.02

Notes: TS = Total Solids; TVS = Total Volatile Solids; EO = Essential Oils; OP = orange peel; PM = poultry manure.

Table 2 - Characteristics of biogas and experimental conditions (OLR, EOsr) in the anaerobic digestion tests.

	Parameters			Days								
				1-4	***5-7***	***8-10***	***11-13***	***14-17***	***18-21***	***22-34***	***35-39***	***40-43***
CONDITIONS	**Mesophilic**	*OLR [g TVS L^{-1} d^{-1}]*	*OP*	0.64	0.64	0.64	0.64	1.07	1.07	1.98	2.50	1.98
			PM	0.00	0.36	0.00	0.36	0.31	0.00	0.00	0.00	0.00
		EOsr [mg L^{-1} d^{-1}]		28.6	28.6	28.6	28.6	47.6	47.6	88.1	111.2	88.1
		HRT [d]		70.0	70.0	70.0	70.0	42.0	42.0	22.7	17.9	22.7
		*Daily specific methane yield** *[L g TVS^{-1} d^{-1}]*		0.39 ^{aA}	0.49 ^{aA}	0.27 ^{aA}	0.31 ^{aA}	0.68 ^{bA}	1.22 ^{bA}	0.67 ^{bA}	0.04 ^{cA}	0.00 ^{cA}
		CH_4 content in biogas [% v/v]		56.0	57.8	58.4	59.0	60.6	66.3	66.0	33.4	6.9
		pH		7.90	7.90	7.79	7.68	7.71	7.90	7.09	4.77	3.55
	Thermophilic	*OLR [g TVS L^{-1} d^{-1}]*	*OP*	0.64	0.64	0.64	0.64	1.07	1.07	1.98		
			PM	0.00	0.36	0.00	0.36	0.31	0.00	0.00		
		EOsr [mg L^{-1} d^{-1}]		28.6	28.6	28.6	28.6	47.6	47.6	88.1		
		HRT [d]		70.0	70.0	70.0	70.0	42.0	42.0	22.7		

		*Daily specific methane yield** *[L g TVS^{-1} d^{-1}]*	0.11 ^{aB}	0.23 ^{aB}	0.40 ^{bB}	0.28 ^{bB}	0.30 ^{bB}	0.33 ^{bB}	0.05 ^{aB}
		CH_4 content in biogas [% v/v]	19.8	38.0	48.7	53.4	37.4	37.6	11.2
		pH	7.80	8.00	8.00	8.00	8.10	7.4	5.56

Notes: OP = orange peel; PM = poultry manure; OLR = Organic Loading Rates; EOsr = Essential Oil supply rates; HRT = Hydraulic Retention Time; * = referred to mean values. Different lowercase letters indicate significant differences (at $p < 0.05$ of Kruskal-Wallis test) between values within the same experimental test (mesophilic or thermophilic conditions); different capital letters indicate significant differences between mesophilic or thermophilic tests (at $p < 0.05$ of Mann-Whitney test).

Table 3 - Values of Organic Loading Rates (OLR) and Essential Oil supply rates (EOsr) of the anaerobic digestion tests and their effects on the specific methane yield.

CONDITIONS				**Effect on methane production**
Mesophilic		**Thermophilic**		
OLR [g TVS L^{-1} d^{-1}]	*EOsr* [mg L^{-1} d^{-1}]	*OLR* [g TVS L^{-1} d^{-1}]	*EOsr* [mg L^{-1} d^{-1}]	
0.64 - 1.38	28.6 - 47.6	0.64 - 1.38	28.6 - 47.6	*No inhibition*
1.98	88.1	-	-	*Partial inhibition*
2.50	111.2	1.98	88.1	*Total inhibition*

FIGURE CAPTIONS

Figure 1 - Pilot anaerobic digester used for the semi-continuous experimental tests.

Figure 2 – Organic Loading Rates (OLR) and Essential Oil supply rates (EOsr) during the anaerobic digestion tests in (a) mesophilic and (b) thermophilic temperatures (PM = poultry manure).

Figure 3 - Daily methane yield and content on biogas as well as Hydraulic Retention Times (HRT) in the anaerobic digestion tests (a: mesophilic conditions; b: thermophilic conditions).

Figure 1
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Figure 2
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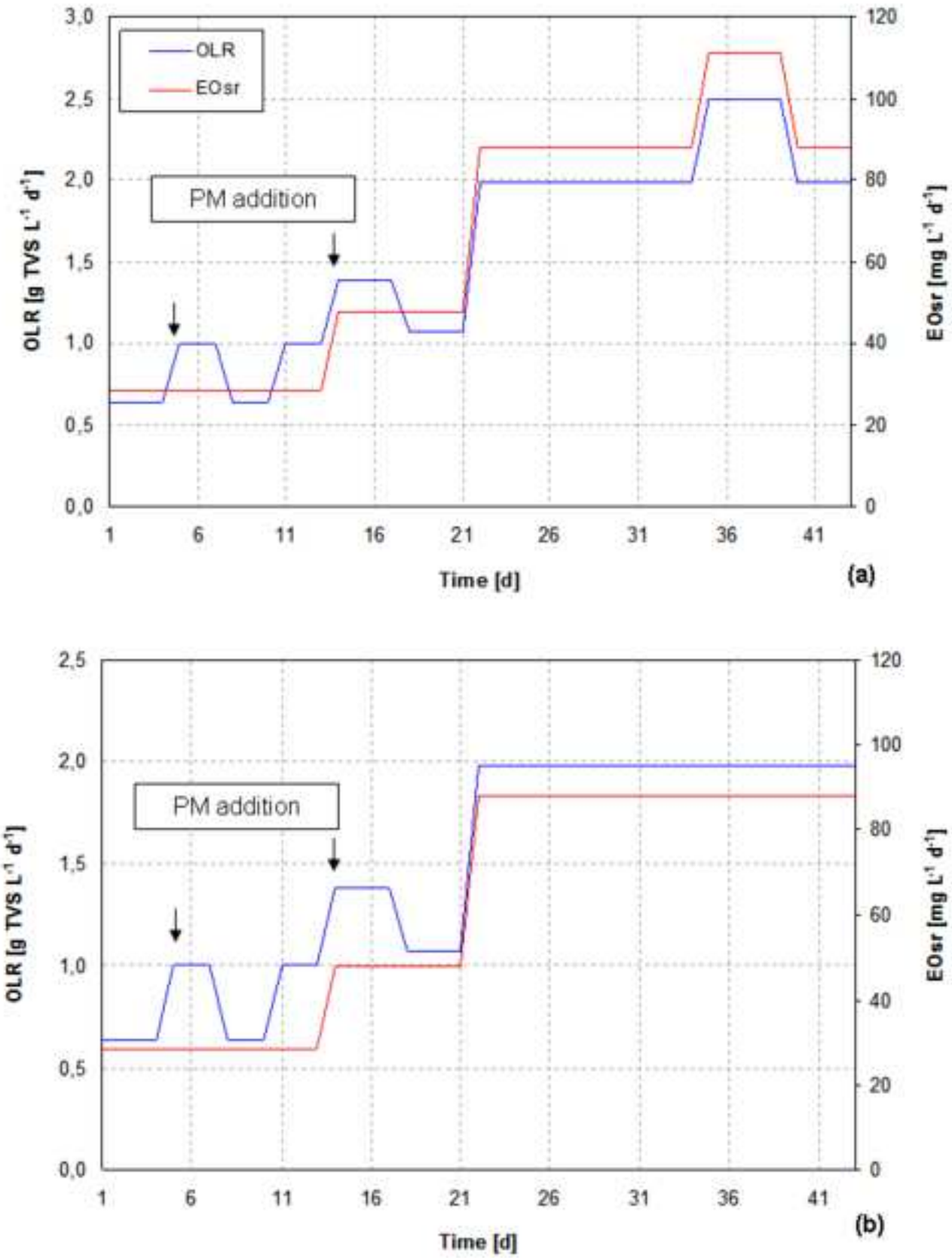

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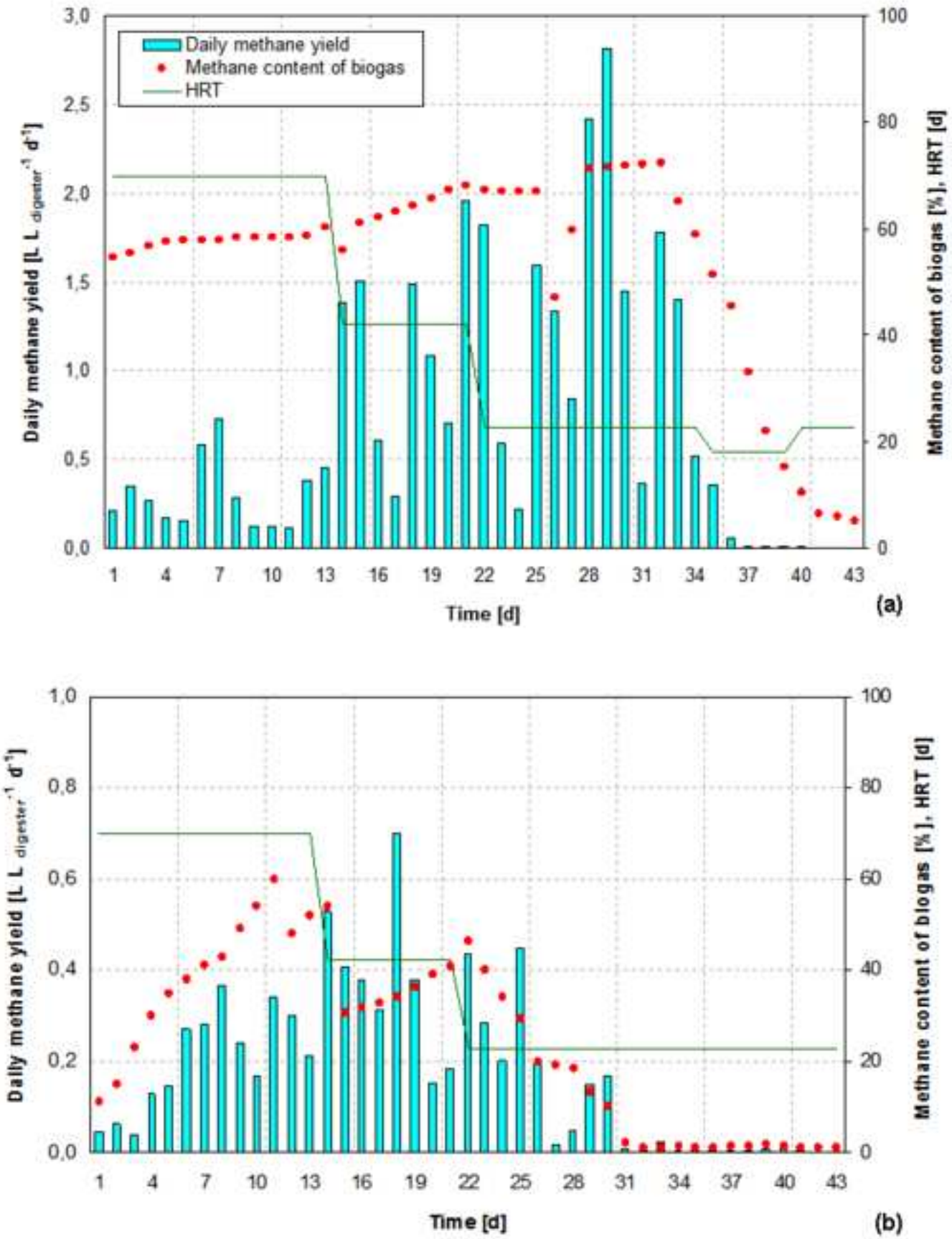

*Author agreement

Anaerobic digestion of orange peel in a semi-continuous pilot plant: an environmentally sound way of citrus waste management in agro-ecosystems

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We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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