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Abstract

This study aims at investigating how organic waste co-digestion coupled with alkaline pretreatment can impact the methane production and fertilizer value of produced digestates. For this purpose, sludge alone and mixed with olive pomace or macroalgal residues were subjected to anaerobic digestion with and without alkaline pretreatment. In addition, co-digestion of pretreated sludge with raw substrates was also carried out and compared to the whole mixture pretreatment. KOH pretreatment enhanced methane production by 39%, 15% and 49% from sludge, sludge mixed with olive pomace and sludge mixed with macroalgal residues, respectively. The digestates were characterized according to their physico-chemical and agronomic properties. They were then applied as biofertilizers for tomato growth during the first vegetative stage (28 d of culture). Concentrations in chlorophyll *a* and carotenoids in tomato plants, following sludge digestate addition, rose by 46% and 41% respectively. Sludge digestate enhanced tomato plant dry weight by 87%, while its nitrogen content rose by 90%. The impact of nitrogen and phosphorus contents in the digestate was strongest on tomato plant dry weight, thus explaining the efficiency of sludge digestate relative to other types of digestate. However, when methane production is considered, the combination of pre-treatment with co-digestion of macroalgal residues and sludge appears most beneficial for maximizing energy recovery and for biofertilizer generation.

Keywords: anaerobic digestion, waste activated sludge, pretreatment, codigestion, biofertilizer, chlorophyll.

Abbreviations

AcoD Anaerobic co-digestion

AD Anaerobic digestion

BMP biomethane potential test

CEL Cellulose

FOS Volatile organic acids

HEM Hemicelluloses

HRT hydraulic retention time

LIGN Lignin

MAR Macroalgal residues

MonoD mono-digestion

NDS Neutral detergent soluble

OP Olive pomace

PLS Partial least square

TAC Total alkalinity

TKN Total Kjeldahl nitrogen

TWAS Thickened waste activated sludge

TS Total solids

VFAs Volatile fatty acids

VS Volatile solids

1. Introduction

As the world population grows, the consumption of food and water is increasing exponentially. Wastewater treatment plants, along with the food industry, generate enormous amounts of organic waste such as sludge, which are, in many developing countries, landfilled or burned (Babayemi and Dauda, 2010). As a country with fairly significant natural resources, Morocco is presently experiencing an increasing generation of biomass waste which needs to be dealt with (Mohamed et al., 2018). Examples of these wastes include olive pomace from oil extraction (OP), red seaweed or macroalgae residues (MAR). Wet olive pomace is generated at 400 000 tons per year (Bouknana et al., 2014), MAR at 870 tons per year (Aboulkas et al., 2017) and dry weight sludge at 255 500 tons per year (Belhadj et al., 2013). The lack in sustainable management of these wastes would eventually lead to long-term harmful effects on the environment, which might even be taking place already (Dahhou et al., 2017). Nowadays, effective waste management strategies are required to ensure the sustainability of the processes and to minimize their impact on the environment. Indeed, currently applied sludge management techniques include incineration, composting, anaerobic digestion (AD) and agronomic valorisation (land application) (Cieślik et al., 2015).

Anaerobic digestion, one of the most widespread solutions for processing organic waste, is a biological process that transforms organic matter into biogas and into a residue called digestate. Digestate can have fertilizing or amending properties. The quantity of methane produced and the quality of the digestate necessarily depend upon the properties of the substrates used. They also rely on the AD conditions or the application of biomass pretreatments. Among these properties, substrate composition and its biodegradability are crucial parameters.

Parameters such as the C to N ratio, moisture content and pH can be adjusted by adding a co-substrate prior to AD, known as co-digestion. Other benefits of co-digestion are the dilution of inhibitory compounds such as fatty acids or phenols but also the improvement of the buffering capacity (Iacovidou et al., 2012). In addition, co-digestion contributes to the balance, in the digesters, between macro and micronutrients that are necessary for anaerobic microorganisms. From a technical point of view, co-digestion allows to take advantage of already existing digesters to manage additional wastes; furthermore, by increasing their energy production the site consumption can be covered (Mu et al., 2020). However, although co-digestion can be applied to improve AD performances, it should be done with caution as there is a risk of modifying the rheology of the mixture and increasing its total solid content (TS) which in turn may reduce the digestor performance (Miryahyaei et al., 2020).

The co-digestion of sludge with other substrates has been investigated intensely during the past decades. The organic fraction of municipal solid waste and food waste can represent two potential co-substrates of the AD process of wastewater sludge. Co-substrates such as fatty wastes and glycerol were also studied, but with sludge/co-substrate ratios generally above 50% (Volatile solids (VS) basis) (Jensen et al., 2014). On the contrary, food wastes or agricultural residues (manure, fruit and vegetable wastes) can be added at a higher ratio but it generally remains around 50% (Algapani et al., 2017). For instance, a 19% improvement in methane production was obtained after co-digestion of sludge with 40% (VS basis) olive pomace (Alagöz et al., 2015). Biomasses such as macro- and microalgae have also been studied as sludge co-substrates but not as often as the other aforementioned wastes (Elalami et al., 2019). For instance, macroalgae (*Ulva spp*.) have been co-digested with mixed sludge at a ratio of 11% (VS basis) but no synergy was observed (Costa et al., 2012). In parallel, Mahdy et al. (2014) observed a synergy for methane production during the co-digestion of the microalgae strain *Chlorella vulgaris*, with primary sludge (+26%), but not with waste activated sludge (Mahdy et al., 2014).

Nonetheless, besides the co-digestion benefit, most of these biomasses are relatively difficult to degrade by AD, as their hydrolysis is the rate limiting step. To overcome this bottleneck, various pretreatment techniques have been applied, with extensive research in the case of sewage sludge (Kor-Bicakci and Eskicioglu, 2019). Hydrothermal pretreatment and steam explosion are among the most effective pretreatments on sludge and lignocellulosic biomasses (Cheah et al., 2020). However, the costs of these pre-treatments raise questions concerning their efficiency (Cheah et al., 2020). More recently, researchers have given more attention to low temperature pre-treatments using a minimum amount of chemicals or by replacing these with organic reagents that are less harmful for the environment (Kamusoko et al., 2019).

Previous works by the authors highlight the potential of alkaline pretreatments for increasing methane production from both olive pomace (Elalami et al., 2020a) and macroalgal residues (Elalami et al., 2020b). Although alkaline pretreatments are not yet used at full scale AD, they favour the enhancement of the sludge methane potential (Heo et al., 2003; Li et al., 2012).

Alkaline pretreatments result in the solubilisation of sugars, polyphenols and lipids while the lignin content in the pretreated OP is reduced (Pellera et al., 2016). In addition, NaOH pretreatment (pH=12, 90°C for 2 h) has resulted in the solubilisation of 67% of crude proteins in sludge (Liu et al., 2008). Alkaline pre-treatment also improves the buffering capacity by adjusting the pH value of the substrate (Kamusoko et al., 2019).

Pretreatment can also enhance the effectiveness of co-digestion. It can be applied to a single substrate as well as to a mixture. However, this approach can generate additional costs, which is why it has been investigated more seldom than co-digestion alone. Examples of pretreatments combined with sludge co-digestion include thermo-alkaline techniques for lignocellulosic residues (Abudi et al., 2016b) and mechanical or thermal treatment for macroalgal and microalgal biomasses (Mahdy et al., 2014; Tedesco et al., 2013). In fact, Abudi et al. (2017) reported that thermal pretreatment of sludge synergistically increased the batch methane production of the mixture containing the organic fraction of municipal wastes, sludge and rice straw at a ratio of 1:1.5:1.5 (Abudi et al., 2016a). In addition, Solé-Bundo et al. (2019) found that thermal pretreatment of microalgae increased the methane production of its mixture with primary sludge (Solé-Bundó et al., 2020).

Pretreatments can further affect the quality of the digestate (Tampio et al., 2016). For example, the KOH pretreatment of wheat straw at a dose of 6% (TS basis) resulted in a 138% higher potassium and 68% higher ammonia content compared to untreated wheat straw digestate (Jaffar et al., 2016). NaOH pretreatment has been the most widely used alkaline pre-treatment in literature (Carrère et al., 2010). However, the substitution of NaOH by KOH can reduce the impact of pre-treatment for the agronomic use of the digestate, since sodium can increase the salinity of the soil (Bolzonella et al., 2018).

Very few studies have yet investigated the impact of pretreatment on plant properties after digestate application. Solé-Bundó et al. (2017) reported that although digestate from thermally pretreated microalgae improved digestate hygienisation , it did not increase the cress growth index (Solé-Bundó et al., 2017).

In a previous study, a comparison was made between digestates originating from untreated and KOH-pretreated macroalgal residues. Both types of digestate led to similar results for tomato and wheat germination and for plant growth. Nevertheless, tomato plant weight increased with the use of digestate issued from the co-digestion of macroalgal residues and sewage sludge, thus implying the advantage of sludge addition to the digestion feedstock (Elalami et al., 2020b). Note that the combination between pretreatment and co-digestion had not yet been investigated.

Sludge pre-treatment and co-digestion have been studied extensively in the field of AD processes and their performances. However, as a novelty, the present study proposes to examine the effect of pre-treatment, either alone or combined with co-digestion, on the quality of digestate. The further use of digestate as fertilizer for plant growth is also explored. Here, various pretreatment and co-digestion strategies were applied to organic wastes in order to achieve both bioenergy (methane) and biofertilizer (digestate) production. Semi-continuous reactors were thus used both to confirm the efficiency of alkaline pretreatment on biogas production and to produce the digestates destined as fertilizers. The various digestates were characterised from a physico-chemical point of view and then tested for their efficiency on tomato growth during the first vegetative stage. Finally, the correlation between dry weight of tomato plants and digestate properties was assessed through partial least square regression.

2. Materials and methods

2.1. Substrates and soil

Macroalgal residues (MAR) from agar extraction (*Gelidium sesquipedale*) were provided by a company located in Morocco. The MAR had a TS of 89% and a VS content of 79% (TS basis). The olive pomace (OP) originated from a traditional olive oil extraction mill in the region of Beni Mellal in Morocco. It contained 97% of TS and 88% (TS basis) of VS.

The dried substrates were dried and grinded with a knife mill (SM 100, Retsch, Germany) using a 4 mm screen size. The thickened waste activated sludge (TWAS) was collected from a wastewater treatment plant located in Narbonne-France. This sludge contained 19% TS and 78% VS (TS basis). A paper mill anaerobic sludge was used as inoculum for running the semi-continuous assay. It was characterised by a TS and VS content of 64 g/l and 45 g/l respectively.

Clay-rich soil used for the experiment was sampled between 10 and 30 cm depth from a farm located in the South of France. This soil was prepared as described in (Elalami et al., 2020b). It contained 1.9% organic matter, a C/N ratio of 8.9, a pH of 8.4, 0.13% total nitrogen, 0.04 g/kg total P_2O_5, 0.18 g/kg K_2O and 0.15 g/kg MgO. The cation exchange capacity (7.8 g/kg) was measured using the Metson method (NF X 31-130).

2.2. Alkali pretreatment

A potassium hydroxide solution (Merck, 35% w/w) was added at a dose of 5 g KOH/100 g TS at 25°C. Mixture of sludge (6.7 g wet weight) and MAR (1.42 g wet weight) or OP (1.17 g wet weight) was pretreated by adding 0.36 ml or 0.35 ml of KOH respectively. Water was then added to achieve a solid to liquid ratio of 2 g VS/100 ml and the mixture was kept under agitation at 100 rpm for 2 d. Similarly, TWAS was pretreated alone to be mono-digested and to be co-digested with untreated MAR or OP. For sludge mono-digestion, 13.4 g of wet sludge was pre-treated with 0.36 ml KOH at 25°C for 2 d, whereas for the co-digestion, 6.7 g of sludge was pretreated with 0.18 ml of KOH using 50 ml of water for 2 d. Finally, 1.17 g of OP or 1.42 g of MAR and 50 ml of water were added. For all chemical pretreatments, the temperature, stirring conditions and the substrate/water ratio were maintained at 25°C, 100 rpm and 2 g of VS/100 ml of water respectively.

2.3. Anaerobic digestion assays

Continuous stirred tank reactors (CSTR) with a working volume of 2.5 l were used for the semi-continuous anaerobic digestion of untreated and alkali pretreated mixtures of MAR and OP with TWAS. The reactors were fed manually once a day, and functioned under mesophilic conditions (37°C) with a hydraulic retention time (HRT) of 20 d and an organic loading rate of 1 gVS/l.d. The homogenisation of reactors was ensured by a continuous magnetic stirring system. The anaerobic digestion lasted for about 4 HRTs (80 d), which is in agreement with the scientific consensus that requires at least 2-3 HRTs ensure the stability of the system. The various conditions investigated at CSTR reactor scales are presented in **Table 1**. The monitoring of reactor performances was carried out according to (Elalami et al., 2020b). Biogas production was measured online using Ritter milligas counters, and biogas composition was analysed by gas chromatography (GC CLARUS 480-Perkin Elmer) as described in (Sambusiti et al., 2012).

Table 1 Various feeding conditions applied in CSTR reactors

2.4. Analysis

The APHA method (American Public Health Association) (APHA, 1998) was applied for measuring total and volatile solids . After KOH pretreatment, the solid and liquid phases of the mixtures were separated with a centrifuge (5430, Eppendorf, Germany) at 7830 rpm for 15 min. The solid fraction was then dried overnight at 105°C. The dried solid phase of the mixtures was subjected to the Van-Soest method in order to determine the contents in Neutral detergent soluble (NDS), hemicellulose (HEM), cellulose (CEL) and lignin (LIGN) (Van Soest, 1963). The term "like" was used here to refer to fractions extracted from the Van-Soest method steps, bearing in mind that sludge and MAR are not lignocellulosic. The C, H, N and S content was measured by elemental analysis using Thermo Scientific FlashSmart analyser, via flash combustion at 950°C. Digestate conductivity was measured according to the NF EN 13038. The digestate content in nutrients and in raw substrates (P, K) was determined with the same experimental protocol as described in (Elalami et al., 2020b).

2.5. Tomato growth test

A plant growth test was performed to validate the agronomic quality of the digestates. After trial preparations, the small pots (500 ml) were placed in a growth chamber (Fitotron, Weiss Gallenkamp, UK), according to the OECD 208 guidelines (2006) under controlled light conditions (16 hours of light (4670 LUX) and 8 hours of darkness), temperature (25°C in light period and 18°C in dark period), humidity (60% under light and 80% in the dark).

The application of the digestates was not only compared with unfertilized soil but also with industrial fertilizer. Thus, a dose of 150 kg N/ha was applied for both the digestate and industrial fertilizer (commercially available ammonium nitrate), while the P concentration in industrial fertilizer was 50 kg P / ha (by addition of triple superphosphate).

Each set of conditions contained 4 pots in which six tomato seeds were sowed. The pots were placed in the growth chamber. Every 24-48 h, the pots were watered by weighing and adding distilled water to reach the initial weight. After 70% of the control seeds germinated, three tomato seeds were left in each pot in order to allow enough space for plant growth for dry weight measurement. After 28 d, the tomato plants were harvested by cutting the stems at ground level. A certain amount of fresh plants was kept aside for chlorophyll content determination. The remaining plants were dried for 48 h at 70°C in a forced-air oven, weighed and analysed. The germination index and dry weight of the tomato plants are given in (**Eq.1 and 2**) (Elalami et al., 2020b).

$$\text{Dry weight (gTS/100 plants)} = \frac{\textit{Dry weight of harvested plants } (70^{\circ}\,C)}{\textit{Number of plants}} * 100 \quad \textbf{(Eq. 1)}$$

$$\text{Germination index (\%)} = \frac{\textit{Final number of seeds that sprouted}}{\text{Number of initial seeds}} * 100 \quad \textbf{(Eq. 2)}$$

Chlorophyll refers to the green pigments present in plant chloroplasts. They have a major role in photosynthesis, which consists in the absorption of light energy in order to convert carbon dioxide and water into carbohydrates and oxygen. Chlorophyll *a* has a blue/green colour, while chlorophyll b is a yellow/green pigment. In addition, plants also contain orange/red-coloured carotenoids that contribute to the photosynthetic system as accessory light energy absorbers and as photo protectants of the photosynthetic apparatus.

The extraction of chlorophyll *a*, chlorophyll *b* and carotenoids was carried out by soaking 0.5 g of fresh biomass in 10 ml methanol (98%) for 5 min. The solution was then centrifuged at 10000 rpm for 15 min (Hettich Zentrifugen Rotanta 460). 0.5 ml of the clarified solution was sampled and diluted in 4.5 ml methanol (98%). The solution was then analysed with a UV–VIS spectrophotometer (Jenway 7315) to determine the absorbance at 470, 652 and 665 nm. These pigments (chlorophyll *a,* chlorophyll *b* and carotenoids) were quantified using the following equations (**Eq.3, 4 and 5**) (Lichtenthaler, 1987):

$$\text{Chlorophyll a } (\mu g/gTS) = \frac{16.7A_{665} - 9.16A_{652}}{m_{biomass}} x10 \quad \textbf{(Eq. 3)}$$

$$\text{Chlorophyll b} (\mu g/gTS) = \frac{30.09A_{652} - 15.3A_{665}}{m_{biomass}} x10 \quad \textbf{(Eq. 4)}$$

$$\text{Carotenoids } (\mu g/gTS) = \frac{(1000A_{470} - 1.63Ca - 104.9Cb)}{221m_{biomass}} x10 \quad \textbf{(Eq. 5)}$$

2.6. Statistical analysis

A t-test was applied to evaluate the significance of the results obtained ($p<0.05$). Partial least squares (PLS) regression is a statistical method for determining the linear relationship between two matrices X (digestate properties) and Y (dry weight of tomato plants). The PLS of experimental data was performed using SIMCA from UMETRICS. A correlation coefficient R^2 was computed to assess the statistical relationship between the two variables. In addition, the root mean square error of estimation (RMSEE) represents the distance between the observed Y variable and the predicted Y variable. The cross-validated coefficient (Q^2) and root mean square error of cross validation ($RMSE_{CV}$) generated from an internal method used by SIMCA to define the accuracy of the prediction were used for validating the model.

3. Results and discussion

3.1. Elemental analysis of feedstocks

First, the composition of the substrates was assessed in order to estimate the benefits in their pre-treatment and anaerobic digestion. The composition of the substrates used in this study is shown in **Table 2**. The phosphorous content was highest in sludge relative to the other substrates, while more potassium was found in olive pomace. The C to N ratio was highest in olive pomace, mainly due to its high lipid and lignin content (Elalami et al., 2018). Conversely, sludge presented the lowest C/N ratio which explains why its co-digestion with other substrates that have a better C/N is more favourable. The optimal C to N ratio for anaerobic digestion is within the 25-30 range (Appels et al., 2008). However, in this study, all mixtures had lower C to N ratios. Anaerobic digestion also depends on the biodegradability of the substrates. Therefore, pre-treatment was applied to increase the biodegradability of the mixtures.

Table 2 Composition of the substrates (sludge, olive pomace and macroalgal residues).

3.2. Impact of pretreatment on Van-Soest fractions

Van-Soest fibres in the raw and pretreated substrates as well as in their mixtures are presented in **Fig.1**. The KOH pretreatment (5% TS basis, at 25°C for 2 d) significantly reduced the hemicellulose-like and cellulose-like fractions contained in TWAS by 70% and 86% respectively, while increasing the easily accessible fractions such as NDS (+163%). This observation concurs with previous studies (Chen et al., 2020; Heo et al., 2003). For example, NaOH pretreatment (10 M added to reach a pH of 12) was found to significantly increase soluble COD, soluble proteins, sugars and volatile fatty acids in waste activated sludge (Chen et al., 2020).

Moreover, the NDS, lignin-, cellulose- and hemicellulose-like composition of the mixtures did not significantly differ from the calculated composition which was based on the sum of the different fractions from the two separate substrates forming each mixture. In the mixture of pretreated sludge and OP, the hemicellulose content fell by 32%, while lignin-like fractions dropped by 28% and NDS rose by 45%. Similarly, a pretreatment of the whole mixture obviously seemed to be more efficient in degrading the most recalcitrant materials contained in both TWAS and OP. The lignin-like content decreased by 53% relative to the untreated TWAS and OP mixture, while the hemicellulose-like fraction fell by 63% and zero effect was observed on the cellulose-like fraction. Conversely, the NDS increased strongly after KOH pretreatments (+91%).

The pretreatment of sludge alone before MAR addition did not have any significant effect on lignin-like and cellulose-like fractions relative to an untreated mixture. However, the strongest effect of alkaline pretreatment could be observed on the whole sludge and MAR mixture, where lignin- and hemicellulose-like fractions dropped by 73% and 64% respectively, while the NDS increased by 71%.

Fig.1 Van-Soest fractions in the substrates, their mixtures, pretreated substrates and mixtures. OP (Olive pomace), MAR (Macroalgae residues), TWAS (Thickened waste activated sludge).

The alkaline pretreatment effect on lignocellulosic biomass such as olive pomace has already been reported in literature. Alkaline pretreatment aims at reducing the lignin content through the cleavage of ester bonds in lignin/phenolic-carbohydrate complexes (Taylor et al., 2011), thus explaining the significant impact of pretreatment of a whole sludge and olive pomace mixture on the solubilisation and lignocellulosic matrix degradation.

It is also noteworthy that neither sludge nor MAR are lignocellulosic matrices. These analysed fractions are only lignocellulose-like fractions that acted as indicators to quantify the impact of pretreatment on organic matter. However, it was obvious that the pre-treatment of sludge alone reduced the hemicellulose-like fraction and enhanced NDS, while the pretreatment of whole mixtures also affected lignin- and cellulose-like fractions. This may be related to the amount of KOH added.

3.3. Anaerobic digesters performance

3.3.1. Methane production

The reactor performances for the different scenarios are presented in **Table 3**. Over a total operating time of 80 d, all reactors were stable by the 50th d (data not shown). According to reactor performance, co-digestion of sludge with OP (R1) and MAR (R4) resulted in increased methane specific production by 75% and 72% respectively relative to R1S, while for co-substrates, co-digestion improved ammonium concentrations and FOS/TAC (Volatile organic acids to total alkalinity ratio). Furthermore, the TS and VS removal increased due to co-digestion of sludge with OP and MAR. This was related to a higher methane production in the co-digesters.

The KOH pretreated sludge (R2S) produced 39% more methane compared to untreated sludge (R1S). The pretreatment of sludge alone and of a whole sludge and OP mixture both showed a similar methane production. Indeed, R2 and R3 produced 13 % and 15 % higher methane volumes compared to R1. This finding might result from an inhibition effect occurring within the R3, probably due to the release of phenolic compounds after delignification of OP by KOH pretreatment, while the R2 functioned normally. For this reason, in similar studies, it is recommended to dose polyphenols in digesters fed with a chemically pre-treated substrate, such as OP, that is rich in lignin. Pellera et al. (2016) observed that the application of a NaOH pretreatment to OP, led to a linear increase in total polyphenol concentrations along with the dose of NaOH, regardless of temperature. they reached 5 mg gallic acid equivalent/gVS at a dose of 1 mmole NaOH/gVS at 25°C for 16 h (Pellera et al., 2016). Note that a phenol concentration of 1.5 g/l should not be exceeded in order to maintain a proper methanogen activity (Monlau et al., 2014).

Hence, alkali pretreatment, applied to sludge only seems to be a more effective and environmentally friendly strategy rather than pretreating a whole mixture of TWAS and OP which requires more KOH addition to the system. Previously, Alagöz et al. (2015) reported that ultrasonic pretreatment applied to waste activated sludge increased the methane potential by 24% during co-digestion with olive pomace, although this was not compared with ultrasonic pretreatment of the whole mixture (TWAS and OP) (Alagöz et al., 2015). The highest methane production was achieved in R6 (fed with alkali pretreated mixture of TWAS and MAR). With a methane yield of 281 Nml CH_4/gVS, the KOH pretreatment enhanced the methane production by 49% relative to the raw mixture (R4). On the contrary, the R5 (fed with the mixture of pretreated sludge and macroalgal residues) did not result in any significant enhancement when compared with the raw mixture.

Table 3 Semi-continuous reactors performance; R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

Some previous studies on sludge, OP and macroalgal residue pretreatments, are presented in **Table 4**. In general, alkali pre-treatments are more effective on lignocellulosic residues (Pellera et al., 2016; Peng et al., 2019; Thomas et al., 2019), since they alter the lignin structure (Cheah et al., 2020).

As reported in (Ruiz-Hernando et al., 2014; Wang et al., 2018), alkaline reagent can increase methane production from sludge up to 34%. Results from the present study agree with this observation. In addition, coupling alkaline pre-treatment with heat has further improved sludge biodegradability and consequently the amount of methane produced. Thermal pre-treatment for 6 hours at 80°C prior to NaOH addition resulted in a 172% improvement in methane production (Zou et al., 2020). For OP, thermal pre-treatment with steam explosion led to a 49% reduction in hemicelluloses, resulting in higher solubilisation and, in turn, increased methane production in the liquid fraction of the pre-treated OP. Nevertheless, alkaline or thermo-alkaline pre-treatment effectively reduce the lignin content of the OP, thus leading to a 17 % and 23% increase in produced methane, as observed by (Elalami et al., 2020a) and (Pellera et al., 2016), respectively. Alagöz et al. (2015) also reported that microwave or US pre-treatment of sludge prior to co-digestion with OP increased methane production by 38% and 44%, respectively (Alagöz et al., 2015). Results from the present study do not achieve this same level of improvement, therefore implying that sludge solubilisation can be enhanced with heating.

As for macro-algae, studies have been carried out on different species of seaweed but never on residues of already industrially exploited macroalgae. In the present study, 49% more methane resulted from KOH pre-treatment of the sludge and MAR mixture. However, to the authors' knowledge, no studies yet discuss pre-treatment of sludge mixtures and macroalgal residues. Generally, thermal or chemical pre-treatments of macroalgae biomass have previously been reported with varying improvement rates ranging between 4 and 26% (Ding et al., 2020; Elalami et al., 2020b; Jard et al., 2013; Vanegas et al., 2015).

The impact of pre-treatment on methane production thus essentially depends on the nature of the substrate used and on its composition in addition to pre-treatment and AD conditions. For this reason, the concept of exergy, has been proposed for comparing different pretreatments (Soltanian et al., 2020). However, future works on exergy and the economic aspects of alkaline pre-treatment still need to be considered.

Table 4 Comparison between the results from the present study and other sludge, olive pomace and macroalgal biomass pretreatment assessments.

3.3.2. <u>Other parameters</u>

The buffer capacity of reactors containing KOH-pretreated substrate was found to be significantly higher than the buffer capacity of reactors containing untreated substrate. Sambusiti et al. (2013) reported that the FOS/TAC ratio should ideally remain lower than 0.3 to avoid inhibition and further acidification of the system (Sambusiti et al., 2013). Here, no inhibition was observed and the reactors exhibited FOS/TAC ratio values ranging between 0.19 and 0.39. The maximum FOS/TAC value (0.39) was achieved in R2. The FOS/TAC ratio fell during the AD experiment, reaching values between 0.13 and 0.24 at steady state. The highest VFA concentrations were found in R2S (0.37 g eq acetic acid/l which is equivalent to 6.2 mol/m^3). This is lower than the inhibitory concentration of 9 mole/m^3 reported in the literature (Appels et al., 2008).

Furthermore, ammonium concentrations ranged between 217 mg/l and 600 mg/l, which lies within the recommended range for anaerobic microorganisms (200-1500 mg/l) (Rajagopal et al., 2013). Maximal ammonium concentrations were obtained in KOH pretreated reactors. These were at 600, 510 and 568 mg/l in R2S, R3 and R6 respectively. Although such increases in NH_4^+ concentrations may result from the degradation of proteins during KOH pretreatment, ammonium concentrations decreased towards the end of the AD process, tending to stabilize at 411, 237 and 278 mg/l for R2S, R3 and R6 respectively. High ammonium concentrations contributed to an increase in alkalinity, which in turn ensures an optimal level of pH between 6.5 and 7.8. Despite the fact that the pH at the inlet was high (up to 12.8) for pretreated substrates which had not been neutralized, the reactors still worked correctly and digestate pH fell within 6.9 and 7.4 at steady state. In addition, KOH pretreatment resulted in the enhancement of VS removal, ranging between 4% and 39%. This result concurs with the study by Monlau et al. (2015) who achieved a VS removal enhancement of 20% following NaOH pretreatment of sunflowers (1 mmole/gTS, 55°C for 24 h).

Although pretreatment conditions were not identical, the KOH concentration (0.9 mmol/gTS) was similar to that of NaOH used in (Monlau et al., 2015). However, for a weaker lime concentration (2.8 g/100 gTS of organic fraction of municipal solid waste, room temperature and 6 h), the VS removal increased by 21% (Torres and Lloréns, 2008). The effectiveness of a pretreatment therefore also depends on the reagent used, on the liquid-solid ratio and on the studied substrate.

To conclude, co-digestion of sludge with OP and MAR led to in an improvement in methane production relative to sludge alone. This is related to the methane potential of the co-substrates which is higher than that of sludge. In addition, alkaline pretreatment had the strongest impact on reactor performance. In particular, co-digestion coupled with pretreatment clearly improved methane production, digestate ammonium concentrations and VS removal. On the whole, an economical assessment and optimisation remains necessary, involving both costs and benefits of biogas production and use of digestate. As examples, (Aghbashlo et al., 2019; Tabatabaei et al., 2020) have reported exergy based economic analyses.

3.4. Digestates properties

Digestate properties are summarized in **Table 5**. Comparison between digestates produced from sludge mono and co-digestion pointed out that co-digestion residues are richer in organic matter (and carbon) which, in turn, should improve the amending value of sludge digestate.

KOH pretreatment reduced the TS and VS content in digestates. This is mainly due to methane improvement and to the consequent decrease in C and H concentrations in digestates. Nonetheless, regarding the fibre content, lignin, hemicelluloses and cellulose-like fractions seemed to decrease after AD. Fibre removal efficiency was strongly related to the nature of the co-substrate as well as its initial fibre composition. Indeed, the lignin-like fraction decreased in the R2S digestate, while the hemicellulose-like fraction dropped in the R3. The cellulose-like fraction in the R6 digestate decreased in comparison with the R4 digestate.

Regarding the nutrient profile, KOH pretreatment increased the conductivity level in R2S, R2, R3, R5 and R6 up to 1370 µS/cm, probably because of the high potassium content in all the digestates following KOH pretreatment. This result agrees with (Elalami et al., 2020b), who observed how the KOH pre-treatment of MAR (5% at 25°C for 2 d) also improved conductivity and reduced the digestate organic matter content. Jaffar et al. (2015) reported that a KOH pretreatment (6% TS, room temperature for 3 d) enhanced the total nitrogen, phosphorus and potassium contents of digestate by 9% ,7% and 138% respectively, in comparison with untreated wheat straw digestate (Jaffar et al., 2016). Nevertheless, in the current study, the total phosphorous concentration did not appear to be significantly affected by KOH pretreatment, with values ranging between 61.6 and 80.8 mg P_2O_5/kg TS for sludge mono-digestion, 43.5-60 mg P_2O_5/kg TS and 42.1-54 mg P_2O_5/kg TS for sludge co-digestion with OP and MAR respectively. While the potassium content in digestate from untreated substrates varied between 9.4 and 24.6 g

K_2O/kg TS, potassium concentrations varied between 65.2 and 194 g K_2O /kg in digestate issued from sludge or pretreated mixtures.

The ammonium content increased due to higher protein degradation following KOH pretreatment. This finding agrees with the study by Zou et al. (2020) in which a thermoalkaline pretreatment (80°C for 6 h then mixture of NaOH and Ca(OH)2 at pH= 12 and 25°C for 24 h) increased ammonia levels by 140% at the end of the AD process, when compared with sludge alone (Zou et al., 2020). Similarly, thermoalkaline pretreatment (140°C, 60 meq NaOH/l for 1 h) tripled the ammonium concentrations in a sludge and food-waste mixture (Lee et al., 2019).

In addition, total nitrogen and sulfur concentrations slightly fell in digestates issued from KOH pretreated substrates. However, as a CHNS analysis was performed on dried digestate, the volatile forms of sulfur did not contribute to the measured value. This was also the case for nitrogen; indeed, the N and total Kjeldahl nitrogen (TKN) differences could be essentially attributed to the volatilization of NH_3 due to digestate drying prior to elemental analysis.

To conclude, when compared to mono-digestion of sludge, co-digestion significantly improved methane production and increased the organic matter content of the digestate. Concurrently, when compared to untreated substrate digestate, alkaline pretreatment also improved methane production and enriched the digestate with ammonium.

Table 5 Properties of tomato plants grown on unfertilized soil (control) and with industrial fertilizer or different digestates. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

3.5. Efficiency of the various digestates on tomato plant growth

3.5.1. Effect on germination and dry weight of tomato plants

The results of tomato growth tests on soil alone, with industrial fertilizer and after digestate application are summarized in **Fig.2**. Note that the germination index was not significantly impacted by digestate quality.

This finding concurs with Solé-Bundo et al. (2017) who reported that diluted digestates from untreated, pretreated microalgae and microalgae co-digested with sludge did not present any significant effect on the germination index of cress seeds. However, at high digestate concentrations (10%), co-digestion proved to be more effective in maintaining a maximal germination index while mono-digestion residues reduced germination by 40% (Solé-Bundó et al., 2017). Similarly, Alburquerque et al. (2012) observed that the germination index of both cress and lettuce improved when pig slurry digestate was applied at a concentration of 1% (Alburquerque et al., 2012). Such a dilution avoids phytotoxicity issues. However, in the present study, digestate dilution was not required, probably due to their low TS, which could be related to the low OLR applied to the digester. This implies that the content in phytotoxic compounds was low.

Fig.2 Germination index (a) and dry weight (b) of tomato plants grown on unfertilized soil (control) and soil fertilized with industrial fertilizer and different digestates. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residues), R6 (pretreated mixture of sludge and macroalgal residues).

On the contrary, the dry weight of tomato plants, after digestate addition, increased under all conditions. Regarding R1S, it rose by 87% compared to unfertilized soil, quite similarly to the effect of industrial fertilizer addition. However, the addition of R2S did not result in a higher dry weight result compared to R1S. Similarly, R2, R3 and R5, R6 digestates did not show a significant difference in dry weight compared to R1 and R3 digestates, suggesting that KOH pretreatment effect was not significant. By comparing co-digestion with sludge mono-digestion residues, sludge digestate was found to be more beneficial for plant growth, probably thanks to its high phosphorous content, especially in the case of co-digestion with OP. Previously, Solé-Bundo et al. (2017) reported that sludge and microalgae co-digestion residues were less phytotoxic than microalgae digestate, thus suggesting that the addition of sludge enhanced the agronomic value of the digestate (Solé-Bundó et al., 2017). In addition, thermally pretreated microalgae (75°C for 10 h) digestate did not show any impact on cress growth. However, in the case of OP digestates, the presence of phenolic compounds may explain the decrease in dry weight of tomato plants, in comparison with MAR co-digestion residues. Besides, orange waste digestate was previously found to strongly reduce the germination rate of ryegrass (-92%) as well as its dry weight (-50%) (Kaparaju et al., 2012). Similarly, the addition of digestate from the co-digestion of olive waste and citrus pulp resulted in a significant decrease in the germination (-90%) of cucumber growth (Panuccio et al., 2019).

3.5.2. Effect on tomato plant properties

The properties of tomato plants, cultivated on the various digestates produced, are provided in **Table 6.** Co-digestion of sludge and OP residues enhanced the C, H, N and pigment contents in comparison with unfertilized soil. Nevertheless, chlorophyll *b* was unaffected neither by digestate nor by industrial fertilizer addition, while the carotenoid content increased following the addition of industrial fertilizer or of each type of digestate (up to +72%). In addition, the R1 digestate increased the chlorophyll *a* concentration by 8% compared to industrial fertilizer, while the other digestates presented similar or even lower pigment contents, as was the case for the R5 and R6 digestates. This is probably related to a decrease in the absorption of nutrients required for chlorophyll *a* production within the plant. This effect can occur if metal concentrations in the digestate are too high. It is therefore recommended to carry out metal analysis of the digestate before its application, especially for digestate containing macroalgae. Indeed, macroalgae are known to absorb and concentrate metals from a contaminated environment (Wang and Dei, 1999). Furthermore, raw sludge digestate proved to be more profitable for enhancing tomato plant properties in comparison with pretreated sludge digestate. Indeed, the pretreated sludge digestate contained less organic matter than untreated sludge digestate.

Reports on the impact of digestate properties on plant composition are scarce in the literature (Alburquerque et al., 2012; Ronga et al., 2018; Y. Wang et al., 2018). Cow manure digestate is known to enrich soil with nutrients, mainly N, P and K, which in turn improves the nutrient content in watermelon fruit (Alburquerque et al., 2012). Similarly, liquid digestate from pig manure can enhance the sugar and protein content in maize plants by 10 % and 12% respectively (Y. Wang et al., 2018) while another digestate from agricultural wastes has been observed to enhance the nitrogen, potassium and phosphorous content in alfalfa leaves by 18%, 17% and 7% relative to industrial fertilizer (Koszel and Lorencowicz, 2015). In contrast, Sortino et al. (2014) did not observe any effect of urban bio-waste digestate on the C and N levels of harvested tomato plants (Sortino et al., 2014). Moreover, Ronga et al. (2018) found that co-digestion residues from maize silage, triticale silage, cow slurry and grape stalks led to a decrease in the aromatic compounds of peppermint and basil plants (Ronga et al., 2018). The effect of a digestate therefore depends on the substrate composition, on AD conditions and on the type of pre-treatment or co-digestion, in addition to plant and soil properties.

Table 6 Properties of tomato plants from different seeding conditions. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

3.6. Correlation between digestate properties and dry weight

A multilinear regression (PLS) was carried out to relate dry weight of tomato (gTS/100 plants) with the main physico-chemical properties of digestate (*i.e* P, TKN, ammonium, S, K, Ash, LIGN, CEL, HEM, NDS). The volatile matter was not included here as it is related to LIGN, CEL, HEM and NDS. However, ash concentrations were incorporated into the model as they contain metals and other nutrients that had not been measured in this study. Therefore**, Fig.3** illustrates the results of this linear regression. A significant regression model was obtained (R^2=0.976, Q^2=0.851). In addition, the RMSEE (0.91 gTS /100 plants) and $RMSEE_{CV}$ (1.84 gTS /100 plants) were low compared to observed data, suggesting that the model provided a good description of the dry weight results (**Eq.6**).

$$Dry\ weight\ (gTS/100plants) = 13.9 + 0.13\boldsymbol{P}(\boldsymbol{g/kgTS}) + 0.05\boldsymbol{TKN}(\boldsymbol{g/kgTS}) + 0.07\ \mathbf{ammonium}(\boldsymbol{g/kgTS}) + 0.19\boldsymbol{S}(\boldsymbol{g/kgTS}) + 0.00043\boldsymbol{K}(\boldsymbol{g/kgTS}) - 0.002\boldsymbol{Ash}\ (\boldsymbol{g/kgTS}) + 0.006\boldsymbol{LIGN}(\boldsymbol{g/kgTS}) - 0.05\boldsymbol{CEL}(\boldsymbol{g/kgTS}) - 0.006\ \boldsymbol{HEM}(\boldsymbol{g/kgTS}) + 0.002\ \boldsymbol{NDS}(\boldsymbol{g/kgTS}) \qquad \textbf{(Eq.6)}$$

The PLS indicated that only the phosphorus, nitrogen and ammonium contents presented significant positive impacts on the dry mass of tomato plants **(Fig.3a).** Indeed, TKN, ammonium and phosphorus were essentially provided by sludge (rich in P) as well as by the KOH pretreatment (which entailed stronger protein degradation and thus a higher ammonium content). It is noteworthy that these results were obtained during the early phases of growth (first 28 d), which probably explains why the impact of hardly assimilable organic matter was not observed on the dry mass of the plants. These results agree with previous studies where the nitrogen and phosphorus contents were found to be growth-limiting elements, especially for the very early stages of growth (Cooke et al., 2005; Malhotra et al., 2018).

In addition, Razaq et al. (2017) reported that both nitrogen and phosphorus affected plant height, root morphology and chlorophyll content (Razaq et al., 2017). In contrast, Iocoli et al. (2019) found that lettuce dry weight was highly related to ammonium nitrogen. They also reported that organic nitrogen is associated with recalcitrant organic structures which are not readily available for plants (Iocoli et al., 2019). However, these results still need to be confirmed on long term assays in field conditions, since results can be strongly affected by the availability of elements, the soil texture, the climate conditions and the type of plant tested.

Fig.3 PLS results linking dry weight of tomato plants to digestate characteristics, a) centred and scaled coefficients, b) predicted versus observed dry weight of tomato plants.

4. Conclusions and future prospects

Co-digestion improves methane production relative to sludge mono-digestion and both types of digestate virtually share the same degree of impact on plant dry weight. This suggests that co-digestion can improve methane production and ensure a P and N supply, since these nutrients correlate strongly with plant dry weight. Alkaline pre-treatment has shown to improve methane production, although it does not seem to affect the growth of tomato plants. Digestate application increased chlorophyll *a* and carotenoid concentrations in tomato plants. However, the alkaline pretreatment of MAR mixtures led to a fall in concentrations of these pigments in tomato plants. In future studies, the impact of digestate application on heavy metals in soil and plants should be investigated over long-term field trials. In addition, the sustainability of integrating pretreatment and co-digestion strategies will have to be addressed for pilot scales including the analysis of economic, environmental and societal aspects.

Acknowledgments

Special thanks to Blandine Schraauwers (technician at the APESA platform) for her technical assistance during tomato growth and for conducting chlorophyll and carotenoid analysis. We would like to thank the OCP Group, INRAE, APESA, and University Mohamed VI Polytechnic (UM6P) for providing financial support for this work (Atlass Project; https://umr-iate.cirad.fr/projets/atlass-ocp).

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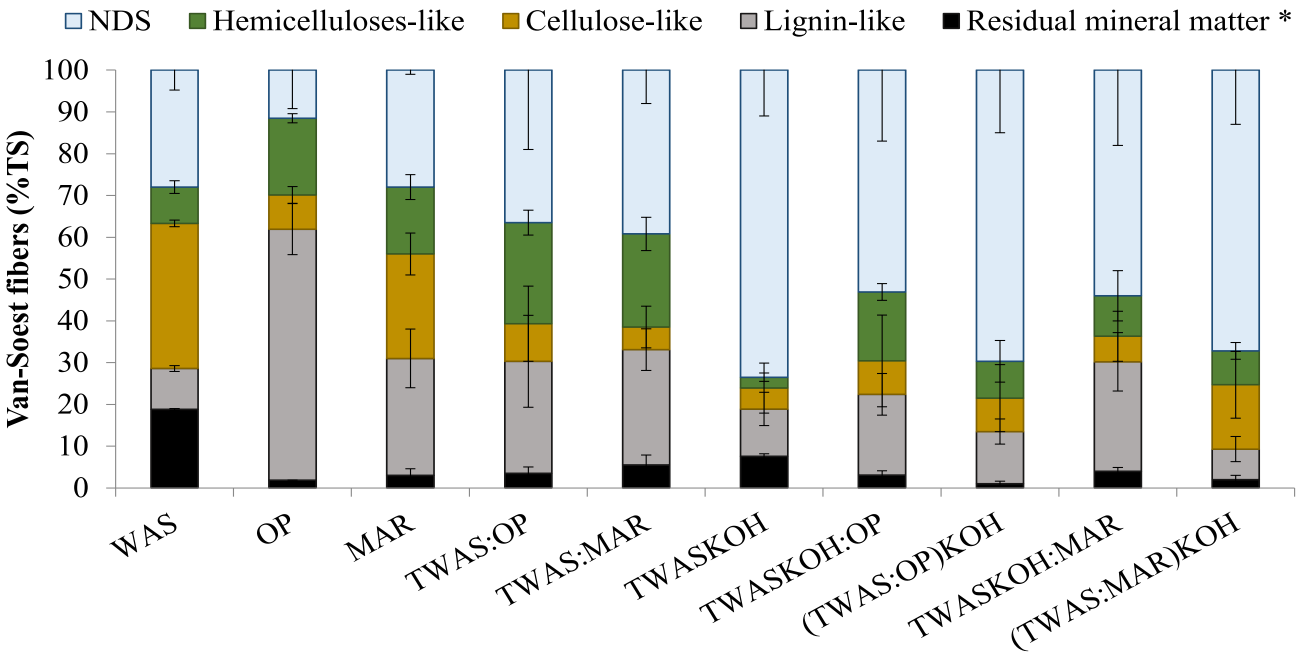
Fig.2 Germination index (a) and dry weight (b) of tomato plants grown on unfertilized soil (control) and soil fertilized with industrial fertilizer and different digestates. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residues), R6 (pretreated mixture of sludge and macroalgal residues).

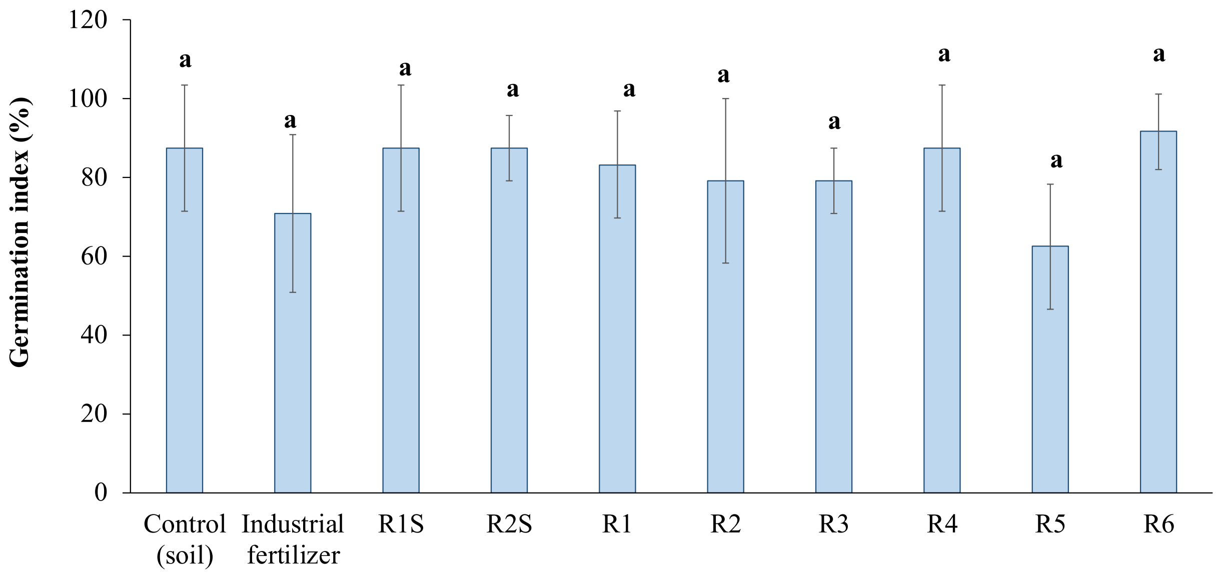
Fig.3 PLS results linking dry weight of tomato plants to digestate characteristics, a) centred and scaled coefficients, b) predicted versus observed dry weight of tomato plants.



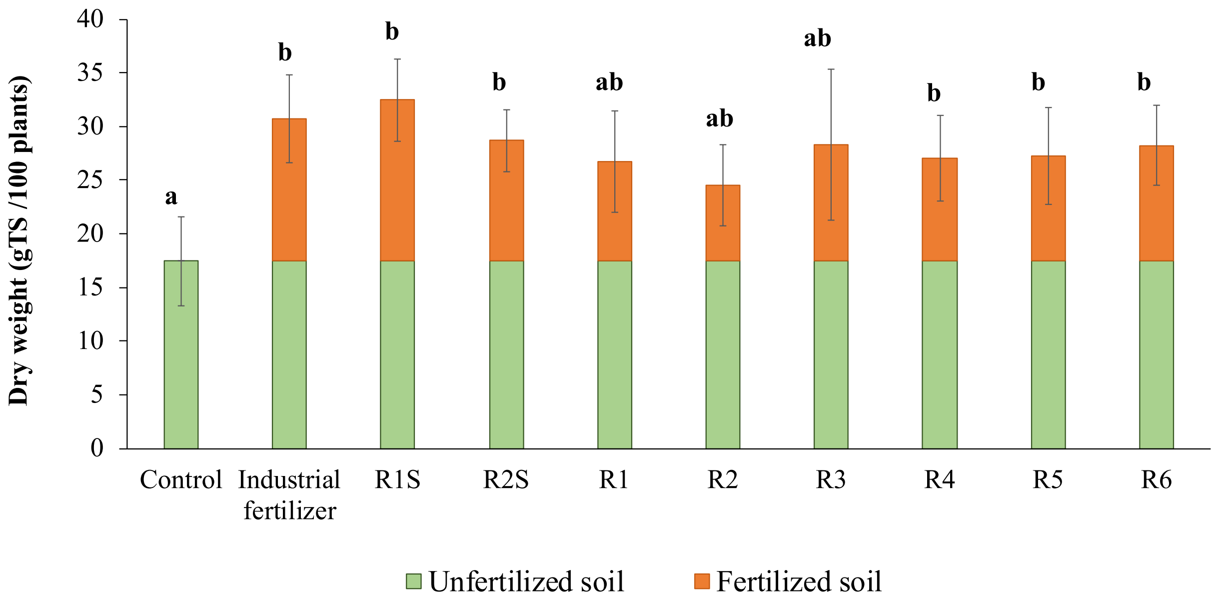
*After burning the "lignin-like" fraction.

Fig.1 Van-Soest fractions in the substrates, their mixtures, pretreated substrates and mixtures. OP (Olive pomace), MAR (Macroalgae residues), TWAS (Thickened waste activated sludge).

a)



b)



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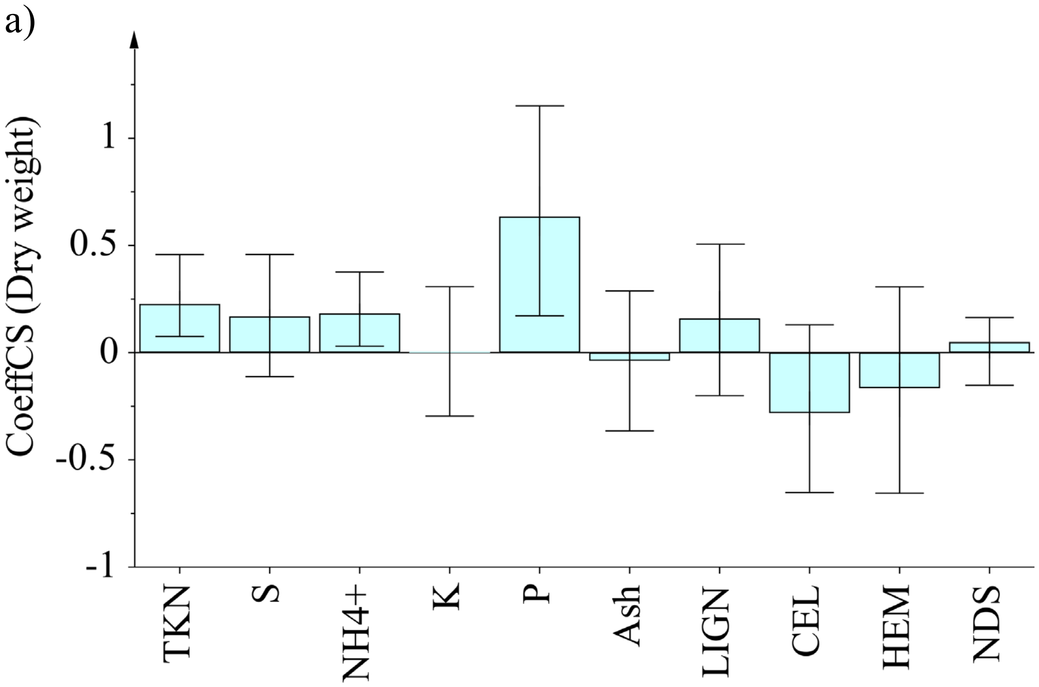
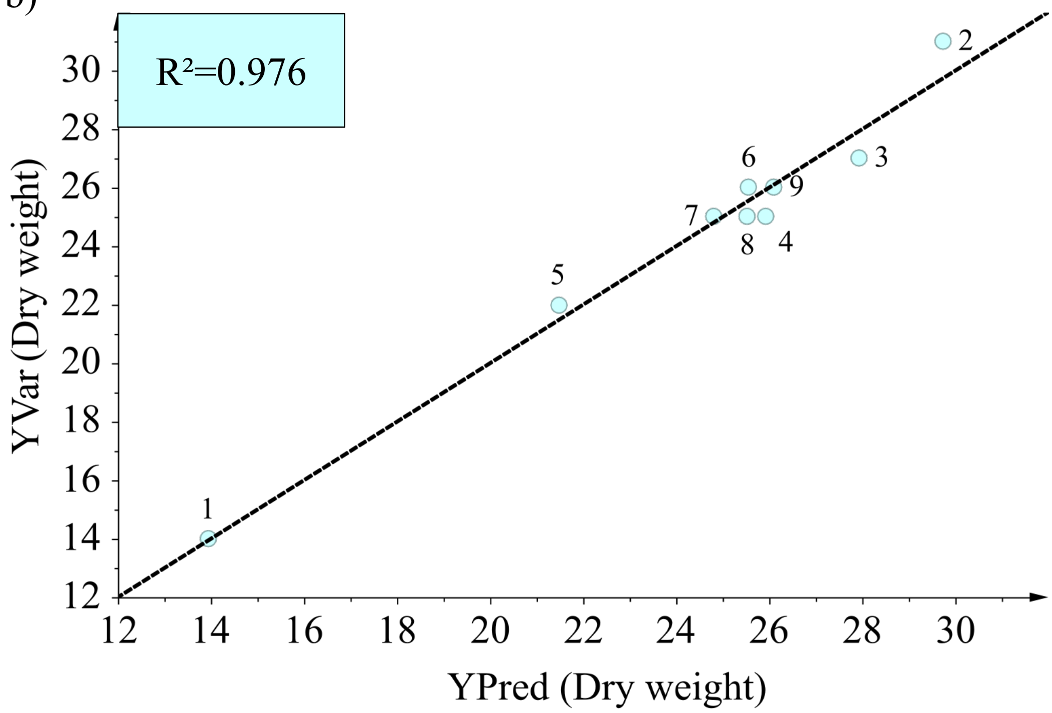
a)



b)



RMSEE=0.907 RMSEcv=1.837

Fig.3 PLS results linking dry weight of tomato plants to digestate characteristics, a) centred and scaled coefficients, b) predicted versus observed dry weight of tomato plants.

Table 1 Various feeding conditions applied in CSTR reactors**.**

	Reactors	**Feedstock**		
		OP (% VS)	TWAS (% VS)	MAR (% VS)
Sludge mono-digestion	R1S-TWAS	0	100	0
	R2S-TWASKOH	0	100 + KOH*	0
Co-digestion	R1-TWAS:OP	50	50	0
	R2-TWASKOH:OP	50	50 + KOH*	0
	R3-(TWAS:OP)KOH	50+ KOH*	50+ KOH*	0
	R4- TWAS:MAR	0	50	50
	R5-TWASKOH:MAR	0	50 + KOH*	50
	R6-(TWAS:MAR)KOH	0	50+ KOH*	50+ KOH*

*Pretreatment conditions: 5% TS, 25°C for 2 d. OP (Olive pomace), MAR (Macroalgae residues), TWAS (thickened waste activated sludge).

Table 2 Composition of the substrates (sludge, olive pomace and macroalgal residues).

Substrates	OP	TWAS:OP	TWAS	TWAS:MAR	MAR
C (% TS)	52±0.2	-	38.3±0.6	-	38.8±0.2
H (% TS)	7.2±0.3	-	5.68±0.05	-	6.1±0.3
N (% TS)	1.0±0.2	-	6.13±0.06	-	4.0±0.2
S (% TS)	0.10±0.01	-	1.12±0.03	-	0.65±0.01
C/N	52.0	12.0	6.7	7.6	9.7
P (g /kg TS)	10.1±0.4	-	39.2±0.4	-	11.5±0.3
K (g /kg TS)	65±4	-	37.3±0.1	-	3.6±0.4
Lipids (%)	16.4±0.8	-	-	-	-

OP (Olive pomace), MAR (macroalgal residues), TWAS (thickened waste activated sludge).

Table 3 Semi-continuous reactors performance; R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

Reactors		pHin	pH at steady state	Methane (Nml /gVSin)	Methane enhancement (% untreated)	TS removal (%TSin)	VS removal (%VSin)	Max VFA (g eq acetic acid/l)	FOS/TAC		NH_4^+ (mg/l)	
									Max	Steady state	Max	Steady state
MonoD	**R1S**	7	6.9	109±5	-	20	28	0.02	0.19	0.13	254	254
	R2S	9	7.5	152±14	39	29	39	0.37	0.29	0.21	600	411
AcoD	**R1**	6.4	7.2	191±10	-	30	37	0.05	0.23	0.21	217	178
	R2	7.2	7.1	215±24	13	32	42	0.18	0.39	0.24	428	217
	R3	12.6	7.2	220±16	15	34	43	0.31	0.31	0.16	510	237
	R4	7.1	7.2	188±19	-	40	46	0.15	0.19	0.14	239	201
	R5	7.3	7.3	194±28	3	38	48	0.3	0.33	0.22	468	257
	R6	12.8	7.4	281±25	49	45	58	0.04	0.32	0.16	568	278

MonoD (mono-digestion), AcoD (anaerobic co-digestion), TS (total solids), VS (volatile solids), VFA (volatile fatty acids), FOS (volatile organic acids), TAC (total alkalinity).

Table 4 Comparison between the results from the present study and other sludge, olive pomace and macroalgal biomass pretreatment assessments.

Substrates	Pretreatment	Conditions	Methane enhancement	Ref
Waste activated sludge	Thermoalkaline	80°C for 6h, then a mixture of NaOH and $Ca(OH)_2$ was added (at a ratio of NaOH: $Ca(OH)_2$ =4:1)	+172%	(Zou et al., 2020)
Waste activated sludge	Alkaline	157 g NaOH/kg TS at 25°C for 24 h. Biomethane potential test (BMP) at 37°C for 35 d.	+34%	(Ruiz-Hernando et al., 2014)
Waste activated sludge	Alkaline	NaOH (pH=8) for 6 d at 35°C. BMP at 35°C for 56 d.	+30%	(Wang et al., 2018)
Olive pomace	Steam explosion	200°C for 5 min and 1.57 MPa. BMP at 35°C for 23 d.	**Untreated OP:** 366 ml/g VS. **Liquid fraction of OP:** 589 ml/g VS. **Solid fraction of OP:** 263 ml/g VS.	(Rincón et al., 2016)
Olive pomace	Thermoalkaline	NaOH (1 mmol/gVS) for 4h at 90°C Biomethane potential test (BMP) at 35°C for 50 d.	+23%	(Pellera et al., 2016)
Olive pomace	Alkaline	NaOH (4%) for 2 d and 25°C BMP at 35°C for 30 d.	+17%	(Elalami et al., 2020a)
Waste activated sludge and olive pomace (1:1 (g/g))	Ultrasonic	200W for 30 min **on sludge alone.** batch-fed anaerobic reactors at 37 °C for 30 days.	+23%	(Alagöz et al., 2015)
	Microwaves	For 30 min at 175 °C and 2000 kPa **on sludge alone.** batch-fed anaerobic reactors at 37 °C for 30 days.	+44%	
Brown macroalga "*Laminaria digitate*"	Thermal	140 °C for 20 min Two-stage anaerobic digestion (batch dark fermentation at 35°C for 3 d, then batch AD at 35°C for 21 d).	+26%	(Ding et al., 2020)
Red macroalga "*Palmaria palmata"*	Alkaline	NaOH (4% TS) at 20°C for 24 h. BMP for 35°C.	+18%	(Jard et al., 2013)
Brown macroalga "*Laminaria digitate*"	Acid	2.5% citric acid 120 °C; 1 h; 1 atm BMP at 35°C for 32 d.	+4%	(Vanegas et al., 2015)
Red macroalgal residues	Alkaline	KOH (5%) for 2 d and 25°C AD CSTR: HRT of 20 d at 37°C.	+20%	(Elalami et al., 2020b)
Sewage sludge	Alkaline	5% of KOH for 2 d and 25°C AD CSTR: HRT of 20 d at 37°C.	+39%	In this study
Waste activated sludge and olive pomace		5% of KOH for 2 d and 25°C **on sludge alone.** Codigestion ratio 1:1 (VS) AD CSTR: HRT of 20 d at 37°C.	+13%	
Waste activated sludge and olive pomace		5% of KOH for 2 d and 25°C Codigestion ratio 1:1 (VS) AD CSTR : HRT of 20 d at 37°C.	+15%	
Waste activated sludge and red macroalgal residues			+49%	

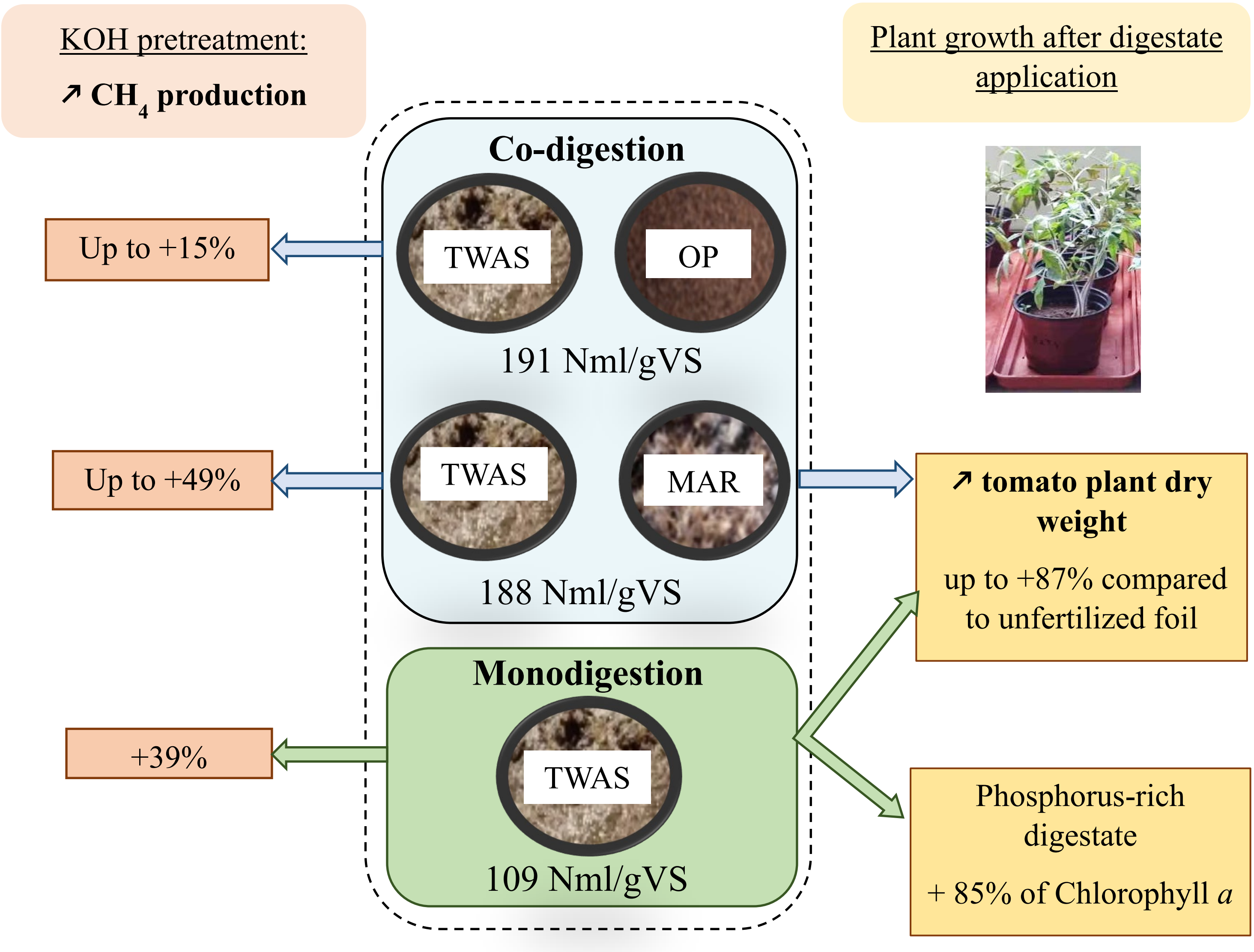
Table 5 Properties of tomato plants grown on unfertilized soil (control) and with industrial fertilizer or different digestates. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

	R1S untreated TWAS	**R2S** TWASKOH	**R1** TWAS:OP	**R2** TWASKOH:OP	**R3** (TWAS:OP)KOH	**R4** TWAS:MAR	**R5** TWASKOH:MAR	**R6** (TWAS:MAR)KOH
Conductivity (μS/cm)	645±1	1370±30	412±3	870±22	1070±35	482±26	684±26	921±28
pH	7.8±0.1	8.3±0.5	7.5±0.1	8.1±0.3	8.2±0.4	7.6±0.0	8.3±0.2	8.3±0.2
TS (%)	1.2±0.0	1.1±0.02	1.2±0.4	1.3±0.03	0.6±0.01	1.0±0.0	0.9±0.01	1.1±0.02
VS (%TS)	70.7±0.1	51±1.2	73.2±0.4	62±1.4	44.5±1.1	74.8±0.1	56.8±0.8	53.8±0.9
Ash (%TS)	29.3	49	26.8	38	55.5	25.2	43.2	46.2
Elemental analysis* (% TS)								
C	34.7±0.1	29.06±0.03	38.7±0.2	34.2±0.1	35.7±0.1	37.2±0.2	33.63±0.03	34.5±0.2
H	6.0±0.2	4.17±0.03	5.7±0.3	4.58±0.01	5.3±0.2	5.2±0.1	4.75±0.08	4.5±0.1
N	4.7±0.1	3.0±0.1	3.9±0.3	3.19±0.01	3.4±0.1	5.6±0.2	4.79±0.04	6.6±0.2
S	1.27±0.01	1.06±0.03	0.98±0.01	0.89±0.02	0.96±0.01	1.50±0.01	1.26±0.02	1.01±0.06
Van-Soest fractions (%TS)								
NDS	31±1	40±5	19±4	31±5	37±3	38±11	32±3	41±2
HEM	33±1	36±4	29±2	38±2	16±2	20±3	32±11	26±5
CEL	2±1	2±1	2±1	4±1	8±3	7±4	3.9±0.1	0.3±0.1
LIGN	26±1	15±3	41±2	21±4	35±4	29±3	25±3	28±3
Nutrients profile								
NH_4^+ (g N/kg TS)	21.2±0.1	37.4±1.5	10.8±2.8	16.7±0.9	39.5±2.3	15.6±0.6	28.6±2.1	25.3±1.7
TKN (gN/kg TS)	50.9±1.2	69.1±0.9	34.7±7.6	38.5±0.9	71.7±1.2	46.5±4.9	57.8±0.3	58.2±0.7
K (g K_2O/kg TS)	18.0±0.1	97.8±1.3	24.6±2.3	48.4±2.3	194.0±4.2	9.4±0.6	65.2±0.9	98.2±1.8
P (g P_2O_5/kg TS)	80.8±1.2	61.6±0.6	60.0±5.1	43.4±2.5	45.4±1.3	54.0±0.7	50.3±3.2	42.1±2.6

*Elemental analysis on the solid fraction of the digestate only. TS (total solids), VS (volatile solids), NDS (neutral detergent soluble), CEL (cellulose), HEM (hemicelluloses), LIGN (lignin), TKN (total Kjedahl nitrogen).

Table 6 Properties of tomato plants from different seeding conditions. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

	C (%TS)	H (%TS)	N (%TS)	S (%TS)	Chlorophyll a (μg/gTS)	Chlorophyll b (μg/gTS)	Carotenoid (μg/gTS)
Control (soil)	35.8±0.2^{a}	5.19±0.01^{a}	1.14±0.02^{a}	0.77±0.02^{a}	48.8±9.9^{b}	22.2±13.2^{a}	13.9±2.1^{a}
Industrial fertilizer (100%)	37.6±0.1^{c}	5.29±0.01^{b}	1.63±0.05^{b}	0.485±0.05^{b}	72.7±0.0^{c}	22.2±0.0^{a}	24.3±1.6^{c}
R1S	37.4±0.1^{c}	5.3±0.1^{ab}	2.17±0.05^{c}	0.74±0.05^{a}	90.4±2.2^{d}	27.8±2.0^{a}	23.6±3.5^{bc}
R2S	36.9±0.1^{b}	5.2±0.1^{b}	1.48±0.03^{c}	0.81±0.07^{c}	61.5±3.0^{b}	18.6±1.2^{a}	19.6±0.6^{b}
R1	35.2±0.1^{b}	5.04±0.03^{ab}	2.2±0.1^{ab}	1.12±0.02^{a}	78.7±3.2^{d}	21.7±1.5^{a}	23.9±1.8^{c}
R2	36.5±0.1^{c}	5.3±0.1^{c}	2.0±0.2^{c}	1±0.1^{a}	65.1±1.4^{c}	20.0±1.2^{a}	22.1±0.5^{c}
R3	37.7±0.1^{b}	5.4±0.1^{c}	2.0±0.1^{b}	0.8±0.04^{b}	67.0±3.0^{c}	20.0±0.9^{a}	22.3±0.4^{c}
R4	36.75±0.04^{b}	5.31±0.01^{c}	1.6±0.1^{c}	0.67±0.07^{c}	69.6±1.5^{c}	27.8±2.0^{a}	23.6±3.5^{c}
R5	35.9±0.03^{a}	5.03±0.02^{c}	2.1±0.2^{a}	0.81±0.08^{a}	21.1±0.5^{a}	21.4±0.7^{a}	23.1±1.1^{c}
R6	37.2±0.02^{c}	5.27±0.02^{b}	1.68±0.1^{ab}	0.713±0.03^{ab}	21.5±0.5^{a}	18.3±0.7^{a}	18.5±0.0^{b}


KOH pretreatment:
↗ CH_4 production
Plant growth after digestate application
Co-digestion
TWAS
OP
191 Nml/gVS
TWAS
MAR
188 Nml/gVS
Up to +15%
Up to +49%
Monodigestion
TWAS
109 Nml/gVS
+39%
↗ tomato plant dry weight
up to +87% compared to unfertilized foil
Phosphorus-rich digestate
+ 85% of Chlorophyll *a*