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TiO₂-AgCl based nanoparticles for photocatalytic production of phenolic compounds from lignocellulosic residues

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Author Contributions

Panagiotis Tsapekos and Merlin Alvarado-Morales contributed equally to the present work and are co-first authors.

ABSTRACT

Lignocellulosic biomass residues can be used as an interesting resource for the production of biochemicals or sustainable fuels. In this optic, lignin represents an interesting raw material for the production of chemicals, such as aromatic compounds, or fuels. This can contribute in moving away from petroleum-based industries to bio-based industries. The aim of this study was to investigate the application of a photocatalytic process using a commercial solution composed by TiO_2 doped with AgCl for wheat straw valorization. The study investigated the effect of pH and catalyst concentrations for optimal phenolic compounds production. It was found that the photocatalytic treatment boosted the phenolic production from wheat straw. The efficiency of the process depended on the initial pH and catalyst concentration. Process optimization towards increased phenolic compounds production was performed. Catalyst concentration of 1 g/L and a treatment time of 1.56 hours in alkaline conditions resulted in the highest phenolics production. Use of the photocatalytic treated wheat straw for anaerobic digestion (AD) was also evaluated. Results showed that the AD microbiome is strongly inhibited by the presence of toxic compounds presented in the catalyst-straw solution and specifically, HNO_3 was toxic to methanogenic communities. Hence, to succeed in an efficient biorefinery framework where total phenols and biogas production are combined, the usage of HNO_3 for catalyst synthesis should be avoided.

1. INTRODUCTION

The concept of sustainable development is strongly related to the view of circular economy.^{1,2} As a matter of fact, the valorization of residues derived from lignocellulosic biomasses for the production of chemicals and fuels represent an interesting option for utilization of this huge resource which currently is only poorly utilized.³⁻⁶ Lignocellulosic biomass residues, such as landscape grass, straws or pruning residues, are chemically composed by a complex ligno-carbohydrate matrix made of cellulose, hemicellulose and lignin.^{4,7} Cellulose and hemicellulose are already widely exploited for biogas, bioethanol and biodiesel production due to their chemical

properties.^{8–10} At the same time, the efficient conversion of the lignin fraction, which is typically underutilized for burning to produce heat or electricity, into high-value chemicals still remains a challenge among researchers due to its highly crosslinked and randomly polymerized composition.¹¹

Although the aromatic chains in lignin could represent an interesting source of chemicals, such as aromatic compounds, or fuels, the main factor that makes difficult the lignin valorization is strongly related to the recalcitrant properties of this polymer.¹² To solve this problem, several depolymerization or oxidative treatments have been tested and developed in order to break down the lignin polymeric structure into aromatic monomers, using physical, chemical, physicochemical, biological and mechanical technologies.^{13–17}

Even though the depolymerization or oxidative treatments are able to break down the chemical bonds of the lignin structure and unpack the chemical structure of lignocellulosic biomasses, the high costs related to them and the production of toxic compounds that could inhibit further processing steps are among the major constraints.^{18,19} Thus, the widespread of the oxidative technologies is hindered, thereby making necessary the development of more sustainable treatments able to minimize or avoid these constraints.

According to recent studies, an oxidizing treatment induced by heterogeneous photocatalysis could lead to promising and interesting results concerning the breakdown of the polymeric structure of lignin.^{20,21} The treatment is based on the optometric properties of semiconductor materials to produce hydroxyl radicals once irradiated by light photons.²⁰ The semiconductor material most frequently used is the anatase form of titanium dioxide (TiO₂) due to its high activity, chemical stability, commercial availability and low cost, although the utilization of other semiconductor materials is possible.⁷ Additionally, photocatalysis generally is a process performed in mild conditions, where the main energy source required is the light irradiation.

When applied to lignocellulosic biomasses, photocatalysis can lead to the production of interesting high-value chemicals. Several types of chemicals can be produced such as vanillin, vanillic acid or ferulic acid from raw biomasses like wheat straw^{22–25}; or methanol, ethanol, formaldehyde, formic acid, oxalic acid from kraft lignin²⁶; or ethyl benzene, vanillin, acetovanillone, acetosyringone, syringaldehyde from industrial lignin.²⁷ Moreover, pretreatment by photocatalysis can enhance biodegradability, which can result in increased biogas production.²⁸

Although some studies have been reported on photocatalytic pretreatment of biomass, the process is yet not optimized.^{29,30} Several operational conditions influence the performance of photocatalytic pretreatment. For instance, pH of the aqueous suspension influences the process efficiency having an important role in the formation/inhibition of active hydroxyl radicals.²⁹ Moreover, catalyst concentration is important for an optimal photocatalytic processing. For example, a catalyst concentration corresponding to 2 g/L has been reported in many studies as the upper threshold value for optimal conditions of the photocatalysis.^{21,31}

The selection of the appropriate catalyst is of major importance. Regarding TiO₂, its photocatalytic efficiency can be enhanced through doping with a metal, such as Pt, Ag, Fe, V, Au.^{7,32} After being doped, the photocatalytic process of the heterostructures is based on the mechanism related to the photo-induced charge generation, separation and migration at Ag–TiO₂ interfaces. Metal and TiO₂ establish a Schottky barrier due to dissimilar work functions.³³ At the interface of two materials, electrons will transfer from the material with a low work function (i.e. 4.26 eV for Ag³⁴) to the material with a high work function (i.e. 5.1 eV for anatase type of TiO₂³⁵). In addition, the Fermi energy of Ag is higher than that of TiO₂ due to the larger work function of TiO₂. Thus, electrons transfer from the Fermi level of Ag to that of TiO₂ until reaching equilibrium and then, new Fermi energy level is established. In other words, the Fermi levels of Ag and TiO₂ are tuned to the same value when the heterostructures are created, forming a new Fermi energy level with more free electrons.³⁶ Recent studies have demonstrated that the doping with Ag seems a promising way to

augment the photocatalytic activity of TiO₂ nanoparticles.^{37–39} However, further investigations are still required in order to understand the advantages of TiO₂ doped with silver when applied for lignin polymerization and optimize the whole process.

In the light of the aforementioned, the aim of this study was to investigate the application of photocatalytic process using TiO₂ doped with silver chloride for wheat straw valorization. In more detail, the study investigated the optimal conditions for total phenolic compounds (TPC) production, considering different starting pH and catalyst concentrations. In addition, anaerobic degradability tests were carried out in order to elucidate the suitability of the catalyst-straw solution to be added in the feedstock of AD reactor and investigate possible inhibitors to the microbial community.

2. EXPERIMENTAL SECTION

All chemicals used in this study were purchased from Sigma Aldrich ApS (Brøndby, Denmark) while gases were provided by AGA A/S (Copenhagen, Denmark).

2.1. Substrate and anaerobic inoculum. Wheat straw residues were collected from the surrounding area of Copenhagen (Zealand, Denmark). After arrival into the laboratory, its particle size was reduced to less than 0.5 cm by means of a cutting mill (SM 200, Retsch GmbH, Germany). Subsequently, the resulting fibers were grounded in order to obtain a homogeneous powder size of the biomass using a disc mill (TS 250, Siebtechnik).

The total solids (TS) and volatile solids (VS) of wheat straw were determined to be 928.39 ± 0.01 g/kg and 867.22 ± 3.85 g/kgTS, respectively. The substrate was composed by 420.2 ± 0.72 g/kgTS, 307.5 ± 0.55 g/kgTS, 267.3 ± 2.35 g/kgTS of cellulose, hemicellulose and lignin, respectively. The chemical oxygen demand (COD) and C/N ratio were, correspondingly, 1060.92 ± 43.26 g/kgTS and 103 ± 0.50 . The inoculum used for the AD experiments had a pH of 8.32, TS of 18.85 ± 2.80 g/kg and a VS content of 849.60 ± 69.18 g/kgTS.

2.2. Catalyst. The catalyst was provided by a commercial company (ACT Global, Kastrup, Denmark) in an aqueous solution, specifically designed for oxidizing treatments. The aqueous solution was composed of TiO₂ nanoparticles in anatase form doped with silver atoms at a concentration of 20 g/L for TiO₂ and 1 g/L AgCl. The solution contained HNO₃ at a concentration of 22.65 g/L and 2-propanol at a concentration of 2 mL/L. The pH of the solution was 1.02. The color of the solution in its normal composition was close to transparent, while light absorption, measured by means of a UV-Vis spectrophotometer (Helios Epsilon, Thermo Spectronic®), gave a maximum absorbance (au), at a wavelength range between 325 and 350 nm. Further details about the used catalyst can be found in previous research.⁴⁰

2.3. Photocatalytic experiments. A quasi-collimated beam apparatus equipped with the doped medium pressure lamp (SR HUV700, 700 W) with increased emission at low wavelength was used in this study. A more detailed description of the lamp characteristics and reactor setup can be found in previous study.⁴¹ Samples preparation for the photocatalytic experiments started with the soaking of 0.0958 g of wheat straw powder in 25 mL solution formed by the catalyst solution and distilled water. The ratio between the catalyst solution and water was fixed according to the experimental design that is described below into detail, whereas, when a modification of the initial pH was required, a solution of 10% (w/v) NaOH was added. The resulting solution was then mixed with the aid of a magnetic stirrer in dark conditions for 24 h. Dark mixing was performed in order to ensure that low molecular weight fragments such as monomers and dimers of lignin are dissolved in the aqueous medium.²⁷ After that, 16 mL of the prepared solution were transferred into a cylindrical cuvette and put inside a single-beam UV-Vis spectrophotometer (Spectronic 20D+, Thermo Scientific®) for 3 h. The spectrophotometer was set to a wavelength of 340 nm according to the absorbance characteristics of the catalyst solution, while the cuvette was shaken manually every 20 minutes in order to ensure appropriate distribution and contact between substrate and catalyst. The experiments were carried out at ambient temperature (20° C). Samples of 1.5 mL were taken,

respectively, after the sample preparation (i.e. t_0), after 24 h of dark mixing or in other words, at the initial time of the photocatalytic treatment (i.e. t_{24}), and every hour after starting the photocatalytic process (i.e. t_{p1} , t_{p2} , t_{p3}) and stored at $-20\text{ }^{\circ}\text{C}$ for further analysis. Before storage, the samples were firstly centrifuged for 10 min at 13000 rpm and then, the recovered supernatant was filtered by a $0.45\mu\text{m}$ filter (Agilent®).

2.4. Experimental design. The experimental design was built according to the response surface methodology (RSM). RSM is a methodology that explores the relationships between several explanatory variables and one or more response variables allowing to reveal the optimum experimental conditions by conducting the minimal number of experimental combinations.⁹

The design chosen for the study was the rotatable central composite design (CCD), where the variables have been investigated at three different levels in order to define the optimal treatment conditions. The examined variables were the initial pH and catalyst concentration. The values were set in terms of alpha points. Concerning the pH, the values were 1.5 and 12.0 as lowest and highest, respectively. Concerning the TiO_2 concentration, 1 and 10 g/L on the total experimental solution were examined as lowest and highest limits, correspondingly. The experimental conditions planned for each experiment are reported in Table 1. To define the effect of photocatalysis on TPC over time, all experiments were conducted for a period of three hours and samples were taken every one hour of irradiation. Control experiment prepared with only distilled water and wheat straw without any adjustment of the pH, catalyst addition and light irradiation were also performed. Design-Expert software (version 7.0, Stat-Ease Inc., Minneapolis, Minnesota) was used to build and determine the second-order polynomial model. The model was built in order to describe the interactions between the degradation rate expressed in the higher TPC yield and the examined variables concerning also the whole process time. The reliability of the model was evaluated by the coefficient of determination (R^2), adjusted R^2 , adequate precision, lack of fit and F-test.

Descriptive statistics were conducted for all data; mean values and standard deviations were calculated. The comparison of quantitative variables among the examined treatments was performed by one-way ANOVA, followed by Tukey post hoc test ($p < 0.05$).

2.5. Determination of TPC. The concentration of TPC produced was determined using UV-visible spectrophotometer (Helios Epsilon, Thermo Spectronic®) according to the Folin-Cicalteau method.⁴² A characteristic peak at 725 nm reveals the TPC content after the reaction with the Folin-Cicalteau reagent and Sodium Carbonate. A calibration curve for each measurement was built using Gallic acid as standard and subsequently, a calibration plot was constructed to quantify the TPC content. The molecules produced after photocatalysis in the liquid phase were analyzed and qualified with a Gas Chromatography/mass spectrometry (GC/MS, Agilent 7890A/ 5975C). The analysis conditions follow: a capillary column was used (HP-INNOWAX, 30 m $\times$ 0.25 mm $\times$ 0.25 μ m) at programmed oven temperature of 60 °C, hold for 2 minutes at 60 °C and then ramped up at a rate of 10 °C/minute to 260 °C and hold at this level for extra 10 minutes; the injector was kept at 280 °C in spit mode with spit ratio of 1:2; helium was used as the carrier gas. The mass spectra of the unknown peaks were compared with National Institute of Standards and Technology (NIST) mass spectral database to identify the organic compounds.

2.6. Biochemical Methane Potential (BMP) assays. The influence of the photocatalytic treatment on wheat straw biodegradability was assessed by means of batch AD experiments.⁴³ The tests were conducted in thermophilic conditions (55 ± 1 °C) following the guidelines of Angelidaki et al.⁴⁴ Batch reactors made of glass vessels with a total volume of 118 mL and working volume of 53 mL were used for the test. The photocatalytic treatments were performed in a solution with total volume of 16 mL, composed by 0.6513 g of wheat straw, distilled water and catalyst solution. The followed procedure for the photocatalytic treatments was according to the methods described in the “Photocatalytic experiments” paragraph, which consisted in 24 hours of dark mixing and, subsequently, the photocatalysis treatment. The catalyst concentration and the treatment time was

set depending on the results of the photocatalysis in order to test three different experimental conditions. After the treatment, the solution and 37 mL of thermophilic inoculum were added and digested in the batch reactors. The organic load was 1 gVS/L. A test with untreated substrate was performed, where 0.6513 g of wheat straw, 16 mL distilled water and 37 mL inoculum were digested without adding the catalyst solution. Moreover, Avicel® PH-101 cellulose was used (1 gVS/L) to validate the accuracy of the BMP experiments. Batch reactors only with inoculum and water (blanks) were included to determine the residual methane production from the inoculum. The inoculum was allowed to degas for seven days in an incubator before setting up the BMP tests. Prior to incubation, the batch reactors were flushed with a N₂/CO₂ (80/20% (v/v)) gas mixture, closed with rubber stoppers and aluminum caps to ensure anaerobic conditions. During the incubation period, the reactors were shaken once a day to avoid the development of dead zones.

2.7. Analytical methods. TS, VS and COD were determined according to the Standard Methods.⁴⁵ Regarding, COD, 3.5 mL of sample, 2 mL of 0.025M K₂Cr₂O₇ and 4.5 mL of concentrated H₂SO₄ with Ag₂SO₄ were added in each digestion tube. The tubes were digested for two hours at 148 °C. Subsequently, the digested samples were titrated with 0.035M NH₄Fe(SO₄)₂ and 3-4 drops of ferroin indicator. For the soluble COD (sCOD), the samples were centrifuged and only the supernatant was used. Determination of Klason lignin and structural carbohydrates was performed according to NREL protocol.⁴⁶ The pH was determined with a benchtop pH/ORP meter (FEP20, Mettler Toledo). The volatile fatty acids (VFA) composition was measured by gas chromatography using a Shimadzu GC-2010 with a Shimadzu AOI-20i auto injector, as described in Kougias et al ⁴⁷; while the methane concentration in the headspace of batch reactors was determined using a gas chromatograph (Thermo Scientific™, Trace™ 1310). For the degradability experiments, the methane yields are reported at STP conditions. For scanning electron microscopy (SEM) analysis, the treated wheat straw after photocatalysis was recovered through a

filter (GF/C Whatman® glass microfiber filter), and followed by oven-drying at 50°C for 24 hours. SEM analysis was performed with an FEI Quanta 200 ESEM FEG without any coating.

2.8. Data analysis and statistics. The efficiency (n) of the light irradiation on the experimental preparations has been evaluated depending on the increase in yield of TPC. The following equation has been used for efficiency of the photocatalytic treatment (n):

$$n = \frac{Y_p - Y_{dm}}{Y_{dm}} \times 100 \quad (1)$$

Where the contribution of the photocatalysis represents the increase of the TPC yield (%), due to photocatalysis treatment performed during light irradiation. Y_p corresponds to the highest yield of phenolic compounds founded during the examined (i.e. t_{p1} , t_{p2} , t_{p3}) light irradiation periods (mgTPC/gLignin), while Y_{dm} corresponds to the yield of TPC at the ending time of the dark mixing/starting time of photocatalysis (mgTPC/gLignin).

The yield used in the previous equation (Y_p , Y_{dm}) was determined by the following equation:

$$Y = \frac{\text{TPC}}{\text{Lignin concentration}} \quad (2)$$

Where the *Lignin concentration* (g-Lignin/L) was calculated depending on the lignin content of wheat straw, TPC was expressed as mg phenolics produced per g of lignin used.

Moreover, an evaluation of the hourly TPC production rate (mgTPC/L/h) was performed using the following equation:

$$\text{TPC production rate} = \frac{\Delta Y}{\Delta t} \quad (3)$$

Where ΔY (mgTPC/L) is the difference between the final and initial TPC yield and Δt (h) is the difference between the final and initial time.

One-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference test (LSD, $p < 0.05$) was used to evaluate if any significant differences were observed in experimental

measurements. All statistical analyses were performed using OriginPro 9.0.0 SR2 software (OriginLab Corporation, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of dark mixing. An increase on the TPC in the aqueous phase during dark mixing was observed depending on the initial pH and the catalyst solution concentration (Figure 1). Specifically, higher the initial pH, higher was the final TPC concentration after dark mixing period. On the other hand, concerning the catalyst concentration, a higher effect was observed in the release of TPC when the concentration of titanium dioxide was less than 5.5 g/L. The highest increases of TPC concentration in the aqueous solution were 62%, 244% and 350%, for the alkali experiments 4, 6 and 10, respectively (Figure 1). The TPC yields were in a range from 80 mgTPC /gLignin (21 mgTPC /gStraw) to 20 mgTPC /gLignin (5 mgTPC /gStraw). These data are in line with the results obtained in a study conducted by Nair et al.²⁷, in which a physical mixture of lignin and catalyst was tested obtaining 80 mgTPC /gLignin. However, the results in the present study were obtained at alkaline conditions (pH from 10 to 12) in contrast to the results reported in the work by Nair et al.²⁷, where the photocatalytic treatments were conducted in aqueous medium without adjustment of the pH.

In the present study, the TPC concentration increase after dark mixing was evidently favored by NaOH, which was added to adjust the pH of the solutions towards neutral and alkaline conditions. Indeed, the utilization of NaOH as pretreatment leads to the dissolution of the lignin structure provoking a cleavage of both ester and β -O-4 linkages. This results in the release of phenolic compounds, such as ferulates and coumarates.^{48,49} Similar effects in terms of lignin depolymerization were also found in previous studies conducted by Tsapekos et al. and Antonopoulou et al.^{50,51} On the contrary, TPC production was not favored when the experiments were carried out in acidic or neutral conditions, where NaOH action has been neutralized or influenced by the acidic environment.²⁹

Figure 1. Effect of dark mixing in terms of TPC production under different photocatalytic conditions according to the CCD

3.2. Effects of photocatalysis. After dark mixing, the photocatalytic treatment, and consequently light exposition of the samples, was immediately started. From the beginning of irradiation, the number of radicals responsible for depolymerization of the target molecules increased as well as the activation of the catalyst by the UV source. Approximately 50 % of the linkages in hardwood and softwood lignin are β -O-4 type bonds.⁵² Due to its less recalcitrant nature compared to other bonds (e.g. C-C)⁵², β -O-4 linkage is commonly selected as the focal point for depolymerization. Oxidative depolymerization of β -O-4 linkage to phenolics initiates *via* formation of $\bullet$ OH radicals produced during photocatalysis. $\bullet$ OH radical can react with aromatics rings *via* electrophilic addition to cleave the bond resulting in the substitution of the OH group.^{53,54} The yield and concentration in terms of TPC for each experiment are reported in Table 2 and Figure 2, respectively. In contrast with the experiments conducted in neutral conditions and with medium or low catalyst concentration (experiments 2, 3, 5, 6 and 11), where no appreciable changes can be found among the highest and the initial TPC yield (i.e. contribution of photocatalysis less than 5%); in experiment 8 there is an evident decrease of the TPC indicating that a degradation occurred. This effect is attributed to the further degradation of phenolic compounds in other products, not detected with the TPC analysis, occurred during the light irradiation period.⁵⁵ Previous studies pointed out that photocatalysis exerts a strong oxidation action and so, the initial products can be further

oxidized.^{20,22,55} For instance, vanillic acid was the major detected molecule (> 90 mg/L) during lignocellulosic biomass oxidation under TiO_2/UV based photocatalysis.⁵⁶ Specifically, ferulic acid which is covalently linked to lignin molecules and polysaccharides by ester bonds at the cell walls can undergo a first oxidation step to yield vanillin as intermediate compound and then, a further oxidation of vanillin to yield vanillic acid. On this concept, recent studies have proposed the pathway where the most important intermediates from the photocatalytic degradation of ferulic acid identified were: homovanillic acid, vanillyl mandelic acid, trans-caffeic acid and organic acids such as formic acid, acetic acid and oxalic acid. However, the intermediate compounds can be further oxidized to CO_2 and H_2O without yielding any high added value products.⁵⁵ Thus, tailor-made photocatalysis for the selective oxidation of lignocellulosic residues to the targeted products is demanded.

On the other hand, considering the experiments 1, 4, 7, 9, 10, where a medium and high concentration of catalyst was tested, an opposite effect of photocatalysis was observed (Table 2). Particularly, in these experimental conditions a significant ($p < 0.05$) increase of the TPC production after photocatalysis was achieved, highlighting the importance of an adequate catalyst concentration for a proper functioning of the photocatalytic treatment.

Nevertheless, the yield achieved in the experiments 1, 4, 7, 9 was lower than the yield achieved in alkaline conditions and with a medium TiO_2 concentration (experiment 10). This confirms a role of pH for an improved TPC yield in the entire process. Specifically, the pH of the solution has an impact on the generation of OH radicals and the solubilization of lignin. As the pH increases, the presence of large quantity of OH^- ions in the surface of catalyst would enhance significantly the generation of highly oxidative $\bullet\text{OH}$ species by scavenging photogenerated holes.⁵⁷ An increase in the generation of $\bullet\text{OH}$ would contribute synergistically towards the increment of reaction kinetics. Apart from $\bullet\text{OH}$ generation, the alkaline condition could solubilize most of the lignin components into water. Thus, a significantly increased contact between lignin and catalyst is achieved.²⁷ As

addressed in section 3.1, the presence of low molecular weight phenolic fragments in the liquid phase is favoured as a result of the alkaline conditions in the reaction medium.^{23,24,58–60} In fact, in experiment 10, the highest achieved TPC yield was 24.69 mgTPC/gStraw (94.6 mgTPC/gLignin) which resulted definitely higher than the alternative experimental conditions. However, only almost 10% of the total lignin was converted into phenolic compounds, indicating that the process should be substantially improved in order to increase the TPC yield. Phenolics produced in experiment 10 where the highest TPC achieved were qualified with the GC/MS instrument. Since the wide different range of phenolics produced during the reaction, it was challenging to detect and quantify all of them separately in the low detected concentrations. In the present study, vanillin, 2-methoxyphenol, vanillic, p-coumaric and ferulic acids were mainly detected. In accordance, the aforementioned compounds were also reported in previous studies.^{54,61}

Furthermore, the SEM images of wheat straw before and after photocatalysis at the experimental conditions 1 and 10 were obtained in order to visualize the effects on structural changes (Figure 3). As can be observed from the SEM image of untreated sample (Figure 3a), the material surface was intact and compact. On the contrary, after photocatalytic treatment at experiment 1 (Figure 3b), in which the higher TPC yield due to photocatalysis was achieved; wheat straw underwent partially structural changes, especially in terms of the outer layer that protects the structure from potential rupture. However, in experiment 10 (Figure 3c), the changes became more significant; suggesting that alkaline condition followed by photocatalysis could disrupt more efficiently the external part of wheat straw. More improvement could be achieved by optimizing the conditions for the generation of active radicals which would specifically target the necessary oxidation steps during the photocatalysis. In this sense, a different reactor configuration, changing the mixing and recirculation system of the photocatalytic system, can be a solution in order to enhance light penetration and the photocatalytic activity.⁴¹

Figure 2. TPC yield (mgTPC/gLignin) under different photocatalytic conditions according to the CCD

Figure 3. SEM images of wheat straw, untreated(a) and pretreated after experiment 1 (b) and 10 (c)

3.3. Optimization of the photocatalytic treatment. The experimental results from the photocatalytic experiments were further analyzed in order to define the optimal treatment conditions to maximize the TPC with respect to the examined variables. The fit summary output supports that the model was reliable, since the F-value was greater than the calculated, respectively of 10.37 ($p < 0.0001$). The model, obtained by a stepwise regression, has a R^2 and adjusted R^2 of 0.93 and 0.84, respectively. The precision expressed as the signal to noise ratio, was 10.66, which was considered adequate.⁶²

The results from the ANOVA pointed out that the significant parameters that affect the TPC yield are the pH, the square of pH and the square of the time. Nevertheless, the most significant

parameters are the pH and the square of the pH. According to the ANOVA, the final equation that describes the model is:

$$\begin{aligned} \text{TPC yield} = & + 57.94 - 15.11 * \text{pH} - 4.46 * \text{Catalyst Concentration} \\ & + 29.32 * \text{Time} + 0.06 * \text{pH} * \text{Catalyst Concentration} + 1.46 * \text{pH}^2 \\ & - 9.52 * \text{Time}^2 \end{aligned} \quad (4)$$

Where the TPC is expressed in mgTPC/gLignin, Catalyst concentration in g/L, Time in h.

The model response of the photocatalytic treatment is depicted in Figure 4, in which the yield of TPC are reported depending on the initial pH and the catalyst concentration with respect to irradiation time. The Figure 4 portrays the TPC production at the four examined treatment times (0, 1, 2, 3 h), calculated according to the Equation 4.

Figure 4. Response graphs of TPC optimized yield as a function of catalyst concentration (g/L) and pH at different examined treatment durations: (a) 0h; (b) 1h; (c) 2h; (d) 3h

The response graphs differ visually pointing out the different effects of the examined variables.

Based on the results of the model, it can be observed that the highest TPC production yields tend to increase within 2 h of treatment at alkaline conditions and lowest catalyst concentration.

Subsequently, at the third hour the TPC concentration tends to decrease indicating that a mineralization of the phenolic compounds occurred, according to the mechanism described in the previous chapter. Thus, according to the computed model, a proper strategy to maximize the photocatalytic process involves the setting of alkaline condition with catalyst concentration of 1.0 g/L and 1.56 h irradiation, where a TPC concentration of 106.24 mgTPC/gLignin can be achieved. These values are in line with previous studies related to the photocatalysis with TiO_2 ^{25,29,63}, in which was suggested that catalyst concentration should not exceed a value of 2.0 g/L in order to avoid light absorption issues.

The present analysis is the first step to optimize the TPC production from lignocellulosic biomass during advanced photocatalytic oxidation. It should be mentioned that the presented model cannot be used for modelling alternative experimental designs and it solely covers the experimental conditions tested in the current study. Further investigations should be carried on in order to validate the model and test additional parameters such as substrate concentration.

3.4. Effect of the catalyst solution on anaerobic biodegradability. With respect to circular economy, the residual lignin-based residues after the production of high added value aromatic compounds can be further utilized for alternative applications. On top of this, pretreated wheat straw could be alternatively used for bioenergy production. Thus, a set of biodegradability experiments was conducted in order to examine the potential of using the photocatalytically pretreated biomass for biogas production. Based on the results of the photocatalysis, pretreated wheat straw obtained after selected experimental conditions were subjected to AD tests: the experiment 1, where the higher TPC yield due to photocatalysis was observed; the experiment 3, with the highest concentration of catalyst; the experiment 10, where the highest TPC concentration

was detected (Table 1). The effect of catalyst solution on the different AD steps was evaluated measuring the sCOD concentration (i.e. hydrolysis), VFA accumulation (i.e. acidogenesis and acetogenesis) and methane production (i.e. methanogenesis).

Concerning the sCOD concentrations, negligible differences ($p>0.05$) were detected among untreated straw (1649.30 ± 30.27 mg/L), experiment 1 (1741.61 ± 61.57 mg/L) and experiment 3 (1618.90 ± 66.38 mg/L) treatments. On the contrary, at the experiment 10 (2038.82 ± 104.66 mg/L) significant higher ($p<0.05$) sCOD concentration was detected. The upper concentration is attributed to the greater amount of TPC generated during the photocatalytic treatment, which has augmented the sCOD of the sample.⁶⁴ The results confirm that a good hydrolysis rate of the substrate occurred since no inhibition can be found.⁶⁵

Monitoring of the VFA showed that especially propionate concentrations were high (Figure 5). Propionate was found in significantly ($p<0.05$) higher concentrations (183 ± 8 mg/L) in the untreated samples compared to the three photocatalytic samples (<66 mg/L). Propionate is a product of acidogenesis and it is further oxidized to acetate, hydrogen, and bicarbonate by non-methanogenic organisms when balanced methanogenic conditions are provided.^{66,67} The high propionate concentration could indicate that lignin-based substrate supports acidogenic propionate forming bacteria, while acetogenic degradation of propionate to acetate is less promoted. Apart from acetate and propionate, the rest VFAs were significantly meaningless and were detected in negligible amounts.

Figure 5. VFA detected in the experimental samples

A strong hindrance to the methanogenic step was detected at all the photocatalytic treated samples (Figure 6). Significantly lower ($p<0.05$) methane production was observed, as the relative production was less than 20% compared to the untreated samples. Hence, subsequent set of batch experiments were conducted to reveal the toxicity thresholds of potential inhibitors. Initially, batch tests using lower concentrations of catalysts solution compared to the initial tests were prepared to define the limit for provoking toxicity to the microbiome.

Figure 6. CH₄ production of the untreated and treated wheat straw

Interestingly, lower CH₄ production was found when the catalyst solution was simply added in all the examined concentrations (Figure 7a) showing a negative effect on AD process. As a matter of fact, the results revealed that the catalyst solution and not the lignin or intermediate products was responsible for the negative effect on CH₄ production. Depending on its concentration, an evident

($p < 0.05$) AD decrement was already notable at a concentration of 10 mL/L. Furthermore, the catalyst caused more than 50% inhibition in the AD community at a concentration of 20 mL/L.

Subsequently, the various components of the catalyst solution were separately added to AD inoculum in order to identify the toxic compounds causing a hindrance of the methanogenic AD microbiome. Testing the single components of the catalyst solution showed that TiO_2 , AgCl and 2-propanol at a concentration of 1000 mg/L, 50 mg/L and 0.10 mL/L, respectively were not causing any adverse effects on the AD (Figure 7b). The results are in agreement with previous studies reporting that TiO_2 and Ag were not toxic at a concentration of 1500 mg/L^{68,69}, which level is higher than the tested conditions. In addition, 2-propanol is a potential substrate for the AD process easily converted to CH_4 .⁷⁰ On the other hand, a strong reduction of the methane production ($p < 0.05$) was found when HNO_3 was used. HNO_3 is an electron acceptor using reducing equivalents to produce N_2 rather than production of methane by methanogens. Therefore, the lower methane production could be explained by a possible alternation of the metabolism of the organic matter for denitrification rather than methanogenesis due to the presence of HNO_3 .⁷¹

Various HNO_3 concentrations were examined in order to reveal the upper concentration of acid that AD community can tolerate. The results clearly showed that presence of HNO_3 was resulting in lower methane production even at low concentrations (i.e. 0.25 mL/L) (Figure 7c). Consequently, the further usage of the photocatalytic stream for AD can be only considered, if the usage of HNO_3 in the preparation of photocatalyst is avoided. For production of appropriate catalysts for AD other acids than nitrate (leading to denitrification) or sulfate (leading to sulfate reduction) should be used. Hence, the present study can pave further the way for an improved photocatalyst' selection process for both TPC and biogas production in an efficient lignocellulosic biorefinery framework.

(a)

(b)

(c)

Figure 7. Relative methane production depending on: (a) catalyst solution concentration (mL/L); (b) different chemicals present in the catalyst solution (mL/L); (c) HNO₃ concentration (mL/L)

4. CONCLUSION

The aim of this study was to investigate the application of photocatalytic process using TiO₂ doped with silver chloride for wheat straw valorization. As emerged from the results, the production of total phenolic compounds (TPC) from lignocellulosic substrates is strongly influenced by the pH and catalyst concentration. It was found that alkaline condition can boost the TPC production. Furthermore, the results revealed that an adequate catalyst concentration for a proper functioning of the photocatalytic treatment is essential. 24 hours dark mixing exerted a positive effect for high added value aromatic compounds production, enhancing the TPC yield more than 80 mgTPC/gLignin. Furthermore, the usage of 5.5 g/L TiO₂-AgCl under alkaline conditions boosted the TPC yield more than 13% within 2 hours of irradiation. Response surface methodology was used to model the interactive effect of pH and catalyst concentration on the yield of TPC precaution. A reasonable agreement of the second-order regression model with the experimental data was found (R^2 and adjusted R^2 of 0.93 and 0.84, respectively). Process optimization focused on TPC yield can be achieved when the photocatalysis is conducted in alkaline conditions, with a catalyst concentration of 1 g/L and a treatment time of 1.56 h. Concerning photocatalytic pretreatment of wheat straw for biogas production, it was found that the presence of HNO₃ in the catalyst negatively affected the process.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. P.T. and M.A-M. conceived the experiment, contributed to laboratory work, wrote and revised the manuscript. D.B. contributed to the laboratory work, wrote and revised the manuscript. M.M. contributed to laboratory work and revised the manuscript. L.G. wrote and revised the manuscript. I.A. conceived the experiment, wrote and revised the manuscript.

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TABLES

Table 1. Experimental design built according to RSM methodology. The experimental label has been categorized depending on the trial conditions: pH-L: acidic pH; pH-M: neutral pH; pH-H: Alkaline pH – TiO₂-L: low TiO₂ concentration; TiO₂-M: medium TiO₂ concentration; TiO₂-H H: high TiO₂ concentration

No. of experiment	Combination (pH – TiO ₂)	pH	TiO ₂ concentration (g/L)	Substrate concentration (gTS/L)	Lignin load (gTS/L)	Catalyst/Substrate
1	pH-L - TiO ₂ -M	1.50	5.50	3.83	1.02	1.43
2	pH-M - TiO ₂ -M	6.75	5.50	3.83	1.02	1.43
3	pH-M - TiO ₂ -H	6.75	10.00	3.83	1.02	2.61
4	pH-H - TiO ₂ -H	10.46	8.68	3.83	1.02	2.26
5	pH-M - TiO ₂ -M	6.75	5.50	3.83	1.02	1.43
6	pH-H - TiO ₂ -L	10.46	2.32	3.83	1.02	0.60
7	pH-L - TiO ₂ -H	3.04	8.68	3.83	1.02	2.26
8	pH-L - TiO ₂ -L	3.04	2.32	3.83	1.02	0.60
9	pH-M - TiO ₂ -M	6.75	5.50	3.83	1.02	1.43
10	pH-H - TiO ₂ -M	12.00	5.50	3.83	1.02	1.43
11	pH-M - TiO ₂ -L	6.75	1.00	3.83	1.02	0.26

Table 2. Yield and contribution of photocatalysis in terms of TPC

No. of experiment	Y_{dm} (dark mixing period) (mgTPC/gLignin)	Y_p (dark mixing + irradiation time period) (mgTPC/gLignin)	Time of Y_p (h)	Contribution of Photocatalysis (%)
1	7.3	14.5	3	98.7
2	11.2	11.3	1	1.5
3	11.1	11.5	2	3.9
4	21.4	23.7	3	10.8
5	13.0	13.1	2	0.7
6	43.4	41.2	3	-4.9
7	5.8	8.5	2	45.5
8	14.4	10.6	1	-26.8
9	8.7	9.8	3	12.9
10	83.8	94.6	2	12.9
11	26.1	25.5	3	-2.0

FIGURES

Figure 1. Effect of dark mixing in terms of TPC production under different photocatalytic conditions according to the CCD

Figure 2. TPC yield (mgTPC/gLignin) under different photocatalytic conditions according to the CCD

Figure 3. SEM images of wheat straw, untreated(a) and pretreated after experiment 1 (b) and 10 (c)

Figure 4. Response graphs of TPC optimized yield as a function of catalyst concentration (g/L) and pH at different examined treatment durations: (a) 0h; (b) 1h; (c) 2h; (d) 3

Figure 5. VFA detected in the experimental samples

Figure 6. CH₄ production of the untreated and treated wheat straw

(a)

(b)

(c)

Figure 7. Relative methane production depending on: (a) catalyst solution concentration (mL/L); (b) different chemicals present in the catalyst solution (mL/L); (c) HNO₃ concentration (mL/L)