

Efficiency of TiO₂ photocatalytic degradation of stevioside in natural aqueous solutions by nested experimental design and mechanism of degradation

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Abstract. The present study deals with the photocatalytic transformation of stevioside, under simulated solar irradiation using titanium dioxide as a photocatalyst. The investigation has involved a study of stevioside decomposition under a variety of experimental conditions, the identification of intermediate compounds, as well as the assessment of mineralization.

A fully nested experimental design was applied to study the effect of various matrices, as well as, the initial stevioside concentration on the variation of the photocatalytic efficiency.

LC-HRMS in positive and negative ESI mode, was brought to bear in assessing the temporal course of the photocatalyzed process. More than one hundred unknown transformation products, most of them in the form of several isobaric species were identified. By employing accurate mass determination, we were able to attribute an empirical formula to each species and through MSn analyses we were capable to characterize the detected transformation products and to distinguish several isobaric species. The overall transformation mechanism was assessed and involved the hydroxylation/oxidation of the molecule and the subsequent loss of the glucoses bound to the parent compound. Acute toxicity of stevioside and its derivatives was evaluated as well using the *Vibrio Fischeri* bacteria

Keywords: Photocatalysis, stevioside, sweeteners, experimental design

1. Introduction

Sweeteners are nowadays considered as emerging contaminants; and due to their high stability under biological, physical and chemical treatment, they are found in natural waters at concentration up to micrograms per liter levels. Among them sweeteners from Stevia the demand of which has nowadays been increased in the food market due to the low-caloric nature of steviol glycosides. Stevioside (St) and rebaudioside-A (R-A) are the two main

steviol glycosides from stevia, which are about 300 times sweeter than sucrose.

Because surface water is the most affected, these sweeteners may first pose a problem to utilities that use surface water as a source for drinking water production. Therefore, a crucial need for more enhanced technologies that can reduce their presence in the environment has become evident. In the last few years, new technologies for the decomposition of organic micro-pollutants in the aqueous environment have been developed. Heterogeneous photocatalysis represents an example of advanced oxidation processes (AOPs) capable of achieving a complete oxidation of large variety of organic and inorganic species, including dyes, pesticides, endocrine disrupting chemicals (EDCs), pharmaceuticals, PPCPs etc. (Calza *et al* 2006). In the present challenging work, TiO₂ photocatalytic oxidation of St was investigated with the scope of determining the degree of reproducibility of the oxidation process under a variety of conditions such as initial substrate concentration, and water matrix, by the employment of chemometrics. For this reason a fully nested experimental design approach was employed to determine the variation of the process with regards to St photocatalytic yield. In analogy with the effects calculated from screening designs these variations give information about the influence of a factor on a response. This information is very essential for photocatalytic studies since from a practical point of view there is a need to investigate the application of TiO₂ for the degradation of the target contaminants at environmental concentrations and at real surface water or wastewaters. Since these matrices are complex, the AOPs have to be carefully operated in order to avoid incomplete mineralization of organic contaminants which may result in the formation of oxidation intermediates which can be more dangerous than the parent compounds.

Another aspect of this work was the identification of possible intermediate products. For this reason powerful analytical techniques such HRMS was employed.

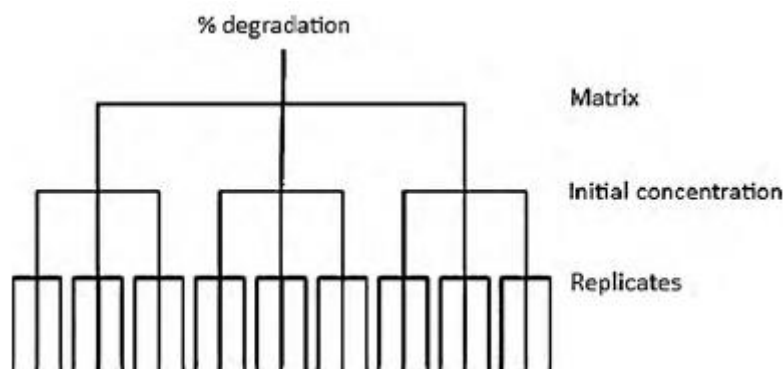


Figure 1. Nested factorial design for photocatalytic degradation

2. Experimental

2.1. Materials and methods

((4 α)-13-[(2-O- β -D-Glucopyranosyl- β -D-glucopyranosyl)oxy]kaur-16-en-18-oic acid β -D-glucopyranosyl ester), (stevioside, ST) with purity higher than 99% was supplied by LGC Promochem Iberia (Wesel, Germany). All solvents used were obtained from Merck (Darmstadt, Germany). HPLC grade methanol (BDH) was filtered through a 0.45 μ m filter before use. Experiments were carried out using TiO₂ Degussa P25 as the photocatalyst. In order to avoid possible interference from ions adsorbed on the photocatalyst, the TiO₂ powder was irradiated and washed with distilled water.

2.2. Irradiation procedures

Irradiation experiments of ST in two natural waters, as well as in distilled water, were carried out on stirred aqueous solutions contained in a cylindrical quartz glass UV reactor (inner diameter 6.0 cm, maximum capacity 75 mL). Degradations were performed on 50 mL of aqueous ST solutions with varying initial concentrations 1, 50 and 100 μ g/L at a fixed concentration of TiO₂ (200 mg/L), according to the experimental design (Table 1). Before irradiation, the suspensions were allowed to stay in the dark for 60 min under stirring, to reach adsorption equilibrium on the semiconductor surface. Irradiation was carried out using a Suntest CPS+ apparatus from Heraeus (Hanau, Germany) equipped with a xenon arc lamp (1500W) and special glass filters restricting the transmission of wavelengths below 290 nm. Chamber and black panel temperature were regulated by pressurized air cooling circuit and monitored using thermocouples supplied by the manufacturer. The light source was on the top of the reactor and an average irradiation intensity of 750 W/m² was maintained throughout the experiments. The temperature of samples did not exceed 20°C using tap water cooling circuit for the UV-reactor.

2.3 Experimental design

In order to calculate the variation of the photocatalytic efficiency (expressed as % degradation), a fully nested experiment of three factors, that is, matrices (distilled water, river water and lake water), ST initial concentration (1, 50 and 100 μ g/L) as well as replicates, was conducted. The design is called nested because the subordinate classification is nested within the higher classification level. The experimental set-up is shown in Fig.1. Matrix was placed in the highest rank of hierarchy being followed by initial ST concentration and replicates, (considering the requirement that factor most affected by systematic effects should be arranged in the highest ranks of the hierarchy and those affected mainly by random effects should be in the lowest ranks of the design). The lowest factor is considered to have a residual variation. The photocatalytic efficiency (response) was calculated for three aqueous matrices of different nature (distilled, river and lake water, $l = 3$). For each matrix, the percentage of photocatalytic degradation was calculated on three concentration levels ($p = 3$), that is, 1, 50 and 100 μ g/L. Finally three replications of each concentration level are considered in the experiment ($r = 3$). Design-Expert software (trial version 7, Stat-Ease, Inc., MN) was used for the data analysis.

3. Results and discussion

The objective of the present nested experiment was to explore the source of variation for the photocatalytic degradation of ST under different conditions. For this reason, a stepwise separate ANOVA was performed in order to assess the variation of the catalytic process for each factor (matrix, substrate concentration, triplicates). These interpretations are based on F-tests, estimation of the variance and expressing these variances relative to the sum of all variances (Vander Heyden *et al.*, 1999). First we consider the variance estimated for each factor as the most interesting. These effects are calculated in such a way that when the effect of a factor is calculated, the influence of all other factors is canceled.

A full model (all effects) considering sources of water, concentration and their interaction was fitted in order to get actual diagnostics and graphs (Fig. 2). As we can observe

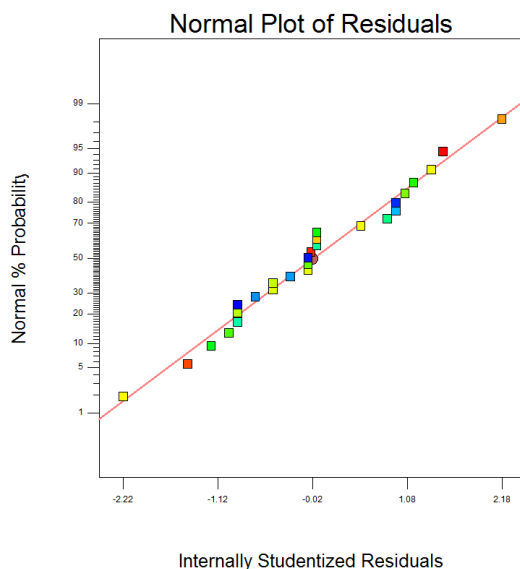


Figure 2. Residual plots for fitted model

from the residual plot, the data were normally distributed indicating a good fit for the model. Besides, when the two mentioned factors (water matrix and ST initial concentration) were considered together and analyzed, then, significant effects with regards to the photocatalytic efficiency was obtained of all model parameters. From Fig. 3 we may observe that the photocatalytic degradation efficiency (% degradation) varied significantly within each water matrix from low to high concentration levels. In general for low initial ST concentration levels (1 µg/L), the degradation percentage was the highest and almost similar for all aqueous matrices. As the initial concentration of ST increases (50 µg/L) a variation of the degradation yield is observed depending on the water type and especially for wastewater. The observation is more pronounced at the highest ST concentration (100 µg/L) at which the photocatalytic degradation of wastewater is remarkably reduced compared to others. As clearly seen, the extent of degradation decreases in the order distilled water > lake water > wastewater and this may be attributed to (i) the increased organic carbon content in lake and wastewater samples (they contain about 10 and 20 mg/L DOC, respectively) which is expected to decrease ST conversion and (ii) the presence of various species (e.g. Cl^- , HCO_3^- , CO_3^{2-}) and other reactive moieties competing for OH \cdot radicals (scavengers) and photocatalyst surface sites.

On the other hand it is clear that the degradation efficiency decreases with an increase in concentration of ST from 1 to 100 µg/L, showing that the degradation rate can be described by pseudo first-order kinetics with respect to the concentration within the experimental range.

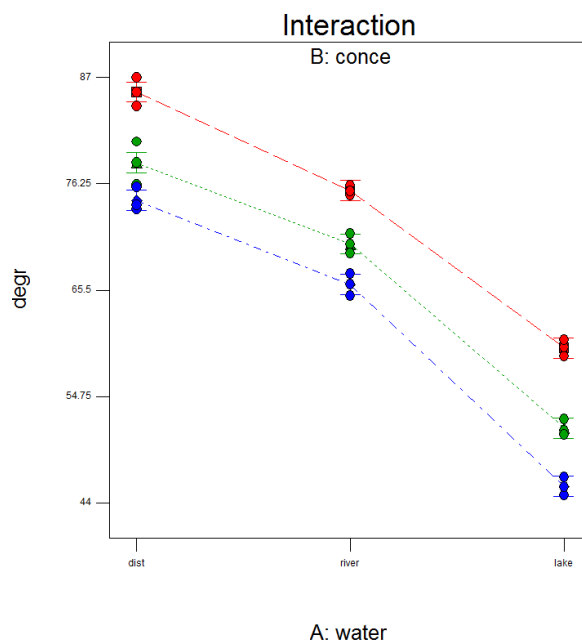


Figure 3. Scatter plot of actual photocatalytic degradation

While there is still an ongoing debate whether photocatalytic oxidation reactions are surface or solution processes, in most cases data processing is performed employing the empirical Langmuir–Hinshelwood kinetic model. Increased concentration of the pollutant could

occupy more active sites of TiO₂, which inhibits generation of the oxidants, reducing the efficiency of the catalytic reaction. Another possible reason is the intermediate products formed upon photocatalytic degradation of ST may compete with the ST molecules for the limited adsorption and catalytic sites on the surface of catalyst particles, and thus inhibit the degradation of ST to a certain extent.

LC-HRMS in positive and negative ESI mode, was brought to bear in assessing the temporal course of the photocatalyzed process. More than one hundred unknown transformation products, most of them in the form of several isobaric species were identified. By employing accurate mass determination, we were able to attribute an empirical formula to each species and through MSⁿ analyses we were capable to characterize the detected transformation products and to distinguish several isobaric species. The overall transformation mechanism was assessed and involved the hydroxylation/oxidation of the molecule and the subsequent loss of the glucoses bound to the parent compound.

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