
# El Colegio de la Frontera Sur

## Capacidad de los hongos de pudrición blanca para degradar el metamizol sódico

TESIS
Presentada como requisito parcial para optar al grado de
Maestría en Ciencias en Recursos Naturales y Desarrollo Rural

por

Rosario Mayorga Santis

2017

**DEDICATORIA**

*A Dios por haberme permitido este logro*

*A mi madre y mi hermana Gabriela por estar siempre conmigo brindándome su apoyo en todo momento*

*A mi padre por convencerme que los propósitos deben estar acompañados de constancia y esfuerzo*

*A mi sobrino Gabrielito*

**AGRADECIMIENTOS**

Al Consejo Nacional de Ciencia y Tecnología (CONACYT) por la beca de maestría otorgada durante el período 2015-2016 con número 623870.

Al Dr. José Ernesto Sánchez Vázquez por la confianza depositada en mí y por permitirme trabajar bajo su dirección.

Al Dr. Rubén Fernando Gutiérrez Hernández por el apoyo incondicional, sus consejos y la disponibilidad de tiempo que siempre mostró.

A la Dra. María De los Ángeles Calixto Romo por su asesoría y valiosas aportaciones en la realización de este trabajo.

A la Mtra. Lilia Moreno por todos sus conocimientos aportados y su paciencia.

Al Mtro. Javier Valle por su ayuda en el análisis estadístico de este trabajo realizado

A mis amigos y compañeros por los momentos vividos que hicieron de esta estancia algo agradable. ¡Gracias por su amistad invaluable!

**ÍNDICE**

## I. Introducción

La fabricación de una amplia gama de productos farmacéuticos como son los analgésicos, los antidepresivos, los antiinflamatorios y los antibióticos para satisfacer las necesidades de salud en nuestra sociedad van en aumento (Yongjun *et al*., 2008; Miceli *et al*., 2014; He *et al*., 2016). La problemática ambiental de estos productos radica en el manejo inadecuado de los sobrantes que no se alcanzan a consumir o bien que llegan a caducar. La fuente principal que libera a estos contaminantes al ambiente es el agua que descargan las industrias farmacéuticas, hospitales y zonas domésticas (Ternes *et al*., 2006; Gomez *et al*., 2007; Kosjek *et al*., 2007; Quesada *et al*., 2009). Dependiendo de las propiedades físico-químicas de los fármacos, estos pueden llegar a alcanzar las aguas subterráneas y contaminarlas o bien quedar retenidas en el suelo y acumularse, pudiendo afectar la vida acuática y a los humanos a través de la cadena trófica (Andreozzi *et al*., 2002; Nash *et al*., 2004; Fent *et al*., 2006; Barceló y López, 2007; Schäfers *et al*., 2007; Gómez, 2011; Watanabe *et al*. 2012).

Esta persistencia y toxicidad en medios acuosos, además de que no se encuentran regulados por ninguna normatividad, ha llevado a considerar a dichos compuestos como Contaminantes Emergentes (CE) (Arrieta y Guerra, 2012)

Existe evidencia de que dichos contaminantes producen impactos como: errores en la muda, eclosión, deformidades anatómicas, cambios subletales en el crecimiento de las plantas, cambios en la proporción sexual de los organismos superiores, modificaciones en los ciclos biogeoquímicos y transmisión de genes de resistencia a antibióticos, variación en el ritmo de vida, reducción en la fertilidad, cambio de la condición sexual por hormonas, efectos tóxicos reproductivos y la muerte (Stuart *et al*., 2012)

Debido a su alto nivel de consumo, en el ambiente se han encontrado grandes cantidades de medicamentos como el ácido acetilsalicílico, el diclofenaco y la dipirona (conocido también como Metamizol), entre otros (Nikolau *et al*., 2007; Martínez *et al*., 2010). El metamizol sódico [(1,5-dimetil-3-oxo-2-fenil-2,3-dihidro-1H-pirazol-4-il) (metilamino)metanosulfonato de sodio] es un analgésico y antipirético de especial uso en hospitales como tratamiento post operatorio. Este tipo de fármacos son administrados en su forma inactiva o significativamente menos activa, y una vez ingeridos son metabolizados *in vivo* al compuesto activo (Pérez, 2008). Posteriormente se hidroliza en su principal metabolito 4-metilaminoantipirina (4-MAA) (Ergün *et al*., 2004), y luego, vía reacciones enzimáticas, en una gran variedad de compuestos como: 4-amino antipirina (4-AA), 4-acetil amino antipirina (4-AAA) y 4-formil amino antipirina (4-FAA) (Kümmerer, 2004; Szabó *et al*., 2013).

Varios estudios realizados demuestran la presencia de sus metabolitos como el 4-MAA, 4-FAA y 4-AAA, en concentraciones de 1486-4304, 3.40-10.1 y 7.30-25.00 $\mu gL^{-1}$, respectivamente, en diferentes descargas de aguas residuales y superficiales (Pérez, 2008; Martínez *et al*., 2007; Feldmann *et al.*, 2008) afectando la flora y la fauna acuática al ser vertidos continuamente en ellos (Andreozzi *et al.*, 2002; Nash *et al*., 2004; Schäfers *et al*., 2007). Si bien la naturaleza tiene una capacidad de biodegradación, también se debe considerar que el aumento de estas descargas de CE, hace más difícil este proceso natural (Miceli *et al*. 2014).

Los procesos avanzados de oxidación (AOPs por sus siglas en inglés), son tecnologías que se basan en la generación in situ de especies transitorias altamente reactivas

($H_2O_2$, ●OH, $O_2$ ●-, $O_3$), para la mineralización de compuestos orgánicos refractarios y eliminación de agentes patógenos (Chong *et al*., 2010), dentro de ellos el proceso Electro-Fenton ha sido evaluado para la degradación de metamizol sódico, obteniéndose valores de remoción medidos en carbón orgánico total (TOC) de 62.8% (Barros *et al*., 2014). Para los metabolitos como el caso del 4-MAA (Pérez, 2008) los métodos empleados han sido la Fotocatálisis con $TiO_2$ y el sistema Foto-Fenton.

Sin embargo, estos tratamientos aunque son factibles presentan inconvenientes como: altos costos de tratamiento, los contaminantes químicos no son destruidos sino simplemente removidos de los efluentes y relocalizados en otro sitio donde el problema persiste (Robinson *et al.* 2001), además que se producen grandes volúmenes de ácidos y de álcalis (Rubilar, 2007).

El objetivo de las plantas de tratamiento de agua residual es la remoción de materia orgánica o sustancias contaminantes a fin de evitar efectos negativos en los cuerpos receptores y lograr que la calidad de este recurso sea la adecuada para las necesidades de los usuarios, así como evitar daño en flora y fauna presente tanto en los sistemas acuáticos como en zonas aledañas a estos. Actualmente, aunque los procesos biotecnológicos para la eliminación de contaminantes ambientales están en pleno proceso de investigación son muy promisorios gracias a que se pueden alcanzar altas eficiencias de remoción de contaminantes y poseen un costo competitivo respecto a tratamientos físico-químicos equivalentes (Robinson *et al.* 2001).

Los tratamientos biológicos son una alternativa ecológica y amigable con el ambiente los cuales consisten en la utilización de organismos, como bacterias y hongos, para la

degradación de la carga contaminante. Estudios previos han mostrado la eficacia de estos procesos en el tratamiento del agua residual contaminada por una gran cantidad de productos químicos y farmacéuticos (Kosjek *et al*., 2007; Ternes *et al*., 2006).

Los mecanismos de degradación que presentan ciertos hongos han sido utilizados para mineralizar diversos medicamentos los cuales provocan efectos dañinos cuando son descargados en aguas superficiales y subterráneas. La actividad enzimática de los hongos de pudrición blanca (HPB) es una alternativa para el tratamiento de aguas que contienen productos farmacéuticos recalcitrantes como son el naproxeno, la carbamazepina, el ácido clofíbrico, el diclofenaco sódico y el ibuprofeno (Marco *et al*., 2010a; Marco *et al*., 2010b; Kartheek *et al*., 2011; Santos *et al*., 2012), los compuestos 1,1,1-tricloro-2,2-bis(4-clorofenil) etano (DDT), 2, 4, 6-Trinitrotolueno (TNT) y Bifenilos policlorados (PCBs) (Ruiz *et al*., 2002; Robles *et al*., 2008; Rodarte *et al*., 2010; Quintero, 2011) y disruptores endocrinos como el 17$\beta$–estradiol y el 17$\alpha$–etinilestradiol (Blánquez y Guieysse, 2008; Lloret *et al*., 2010).

Las enzimas ligninolíticas son relativamente no específicas y utilizan mecanismos de óxido-reducción que permiten la formación de radicales libres los cuáles catalizan la degradación de una amplia variedad de contaminantes ambientales (Pointing, 2001).
Por ello, el estudio de los extractos y las enzimas de los HPB que intervienen en la degradación de diversos compuestos farmacéuticos es un paso necesario para comprender el mecanismo de acción de las enzimas ligninolíticas. Esto permitirá en el futuro desarrollar estrategias de biorremediación.

La pregunta que se plantea entonces es: ¿Puede el extracto enzimático crudo obtenido de alguno de esos hongos de pudrición blanca degradar el fármaco metamizol sódico? Aunado a esto el objetivo del presente trabajo es evaluar la capacidad del extracto enzimático de los hongos *Auricularia fuscosuccinea, Lentinula edodes, Ganoderma lucidum, Agrocybe aegerita, Pleurotus ostreatus, Pleurotus djamor* y *Pleurotus eryngii* en la degradación de metamizol sódico.

## Capítulo de artículo enviado

Carta de recepción del artículo: **Degradation of sodium metamizole by enzymatic extracts from some white rot fungi**. Enviado a la revista SYDOWIA.

SYDOWIA – manuscript submission

Dear Dr. Santis,

we acknowledge receipt of your manuscript "Degradation of sodium metamizole…". The manuscript will now be subjected to critical review. Please be assured that a decision regarding publication will be sent to you at the earliest possible opportunity.

Thank you for considering SYDOWIA in your publication plans.

Sincerely,

Irmgard Krisai-Greilhuber
Chief Editor

Ao. Univ.-Prof. Mag. Dr. Irmgard Krisai-Greilhuber
Department for Botanical Systematics and Evolution
University of Vienna
Rennweg 14
1030 Wien
Austria
Irmgard.greilhuber@univie.ac.at

**********

Chief-Editor of SYDOWIA - An International Journal of Mycology
http://www.sydowia.at/

**Von:** Rosario Mayorga Santis [mailto:romayorga@ecosur.edu.mx]
**Gesendet:** Montag, 27. März 2017 22:37
**An:** irmgard.greilhuber@univie.ac.at
**Betreff:** Manuscript for publication

**Degradation of sodium metamizole by enzymatic extracts from white rot fungi**

R. Mayorga[1], J. E. Sánchez[1*], R. F. Gutiérrez[2] & M. Calixto[1]

*e-mail: esanchez@ecosur.mx

[1]El Colegio de la Frontera Sur, Carr. Antiguo Aeropuerto km 2.5, Tapachula, Chiapas CP. 30700, México

[2]Instituto Tecnológico de Tapachula, Km. 2 carretera a Puerto Madero, Tapachula, Chiapas CP 30700, México

Mayorga R., Sánchez J. E., Gutiérrez R. F., Calixto M. (2017) Degradation of sodium metamizole by enzymatic extracts from some white rot fungi.-Sydowia XX (X): 1-24.

White rot fungi are known for their highly efficient ability to degrade environmental pollutants, such as pharmaceuticals. In this work, the ability of enzymatic extracts from *Auricularia fuscosuccinea*, *Lentinula edodes*, *Ganoderma lucidum*, *Agrocybe aegerita*, *Pleurotus ostreatus*, *P. djamor* and *P. eryngii* to degrade the analgesic drug metamizole was evaluated. The formation of the metabolites 4-methylaminoantipiryne (4-MAA) and 4-amino antipyrine (4-AA) was also monitored. Total degradation of 50 mg $L^{-1}$ of the drug was achieved with the extract obtained from *A. fuscosuccinea* within three days. Under the best conditions (pH 5.6, 35°C and 110 rpm), the degradation profile followed the behavior of pseudo-first order kinetics with a coefficient $k$ = 0.0779 L $mg^{-1}$ $h^{-1}$. The

enzymatic activity tests demonstrated the expression of laccases, lignin peroxidases and phenoloxidases in the degradation process of sodium metamizole. Subsequent studies of degradation of the drug with pure enzymes may indicate whether or not they participate in the degradation.
Keywords: pharmaceutical products, metabolites, ligninolytic enzymes, enzymatic activity, bioremediation, dipyrone.

The manufacture of a wide range of pharmaceuticals, such as analgesics, antidepressants, anti-inflammatories and antibiotics, to meet the health needs of our society is steadily increasing (Yongjun *et al*. 2008, Miceli *et al*. 2014, He *et al*. 2016).

The environmental problem of these products lies in the inadequate management of leftovers that are not enough to consume or that eventually expire. The main source that releases these pollutants into the environment is the water discharged from the pharmaceutical, hospital, and home-based industries (Ternes et al., 2006, Gomez et al., 2007, Quesada et al. 2009).

Depending on the physicochemical properties of the drugs, these substances can reach and contaminate the groundwater or be retained in the soil and accumulate, which may affect aquatic life and humans through the food chain (Andreozzi *et al*. 2002, Nash *et al*. 2004, Fent *et al*. 2006, Barceló & López 2007, Schäfers *et al*. 2007, Gómez 2011, Watanabe *et al*. 2012).

Sodium metamizole [sodium; [(1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl)-methylamino] methanesulfonate] is an analgesic and antipyretic used in hospitals as a post-operative treatment. Once consumed, it is hydrolyzed into its main metabolite, 4-

methylaminoantipyrine (4-MAA) (Ergün *et al*. 2004), which then results in other metabolites, such as 4-amino antipyrine (4-AA), 4-acetylamino antipyrin and 4-formyl amino antipyrin (4-FAA) (Kümmerer 2004, Szabó *et al*. 2013), via enzymatic reactions.

A number of studies show the presence of dipyrone metabolites 4-MAA, 4-FAA and 4-AAA at concentrations of 1486-4304, 3.40-10.1 and 7.30-25.00 μg $L^{-1}$, respectively, in various residual and superficial water discharges (Martínez *et al*. 2007, Feldmann *et al*. 2008, Pérez 2008). Advanced oxidation processes (AOPs), such as Electro-Fenton, Photo-Fenton and Photocatalysis with $TiO_2$, have been evaluated for their ability to degrade metamizole and 4-MAA (Pérez 2008), achieving 62.8% of removal (Barros *et al*. 2014).

Enzymes from white rot fungi (WRF) may be an alternative for the treatment of water containing recalcitrant pharmaceuticals, such as naproxen, carbamazepine, clofibric acid, diclofenac sodium and ibuprofen (Marco *et al*. 2010a, Marco *et al*. 2010b, Kartheek *et al*. 2011, Santos *et al*. 2012). It is also an alternative for the treatment of water containing the compounds 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), 2, 4, 6-Trinitrotoluene (TNT) and Polychlorinated Biphenyls (PCBs) (Ruiz *et al*. 2002, Robles *et al*. 2008, Rodarte *et al*. 2010, Quintero 2011) and endocrine disruptors, such as 17β-estradiol and 17α-ethinyl estradiol (Blánquez & Guieysse 2008, Lloret *et al*. 2010). Ligninolytic enzymes are relatively non-specific and use free radical mechanisms that allow them to catalyze the degradation of a wide variety of environmental pollutants (Pointing 2001). Therefore, studying the extracts and the enzymes of WRF is a

necessary step to understand their mechanism of action and to develop strategies for bioremediation in the future.

The objective of the present work was to evaluate the ability of enzymatic extracts from *Auricularia fuscosuccinea, Lentinula edodes, Ganoderma lucidum, Agrocybe aegerita, Pleurotus ostreatus, P. djamor* and *P. eryngii* fungi to degrade metamizole sodium at the laboratory conditions.

## MATERIALS AND METHODS

### Biological material

Voucher specimens of the evaluated strains *Auricularia fuscosuccinea* ECS-0210, *Lentinula edodes* ECS-0401, *Ganoderma lucidum* ECS-0502, *Agrocybe aegerita* ECS-1009, *Pleurotus ostreatus* ECS-0152, *P. djamor* ECS-0123 and *P. eryngii* ECS-1258 were obtained of the Mycological Collection of El Colegio de la Frontera Sur-Tapachula, Chiapas, Mexico. The substrates used for mycelial growth of the fungi were Pangola grass *Digitaria decumbens*, sorghum *Sorghum vulgare* and shavings of the “Primavera” tree *Tabebuia donnell-smithii*, as indicated below.

### Chemical substances

Malt extract (Bacto™), anhydrous dextrose (HYCEL), casein peptone (MCD lab) and bacteriological agar (MCD lab) were used. HPLC-grade methanol and water were purchased from J. T. Baker and Fermont, respectively. Sodium metamizole, 4-methylaminoantipyrine (4-MAA) and 4-aminoantipyrine (4-AA), all three in standard grade, and sodium acetate, in analytical reagent grade, were purchased from Sigma-Aldrich (Toluca, México). The pharmaceutical formulation of sodium metamizole (500

mg active ingredient, Sanofi-Aventis) was also purchased on the local market and used after purification of the active ingredient.

Purification of metamizole

3.028 g of the pharmaceutical formulation of metamizole sodium were macerated and dissolved in 75 mL of methanol. This mixture was vacuum filtered using Whatman No. 2 filter paper and then evaporated for two hours using a rotary evaporator (Hahn Shin Scientific) at a constant temperature of 75°C and 50 rpm. The recovered crystals (0.750 g) were placed into a desiccator for 12 h to remove residual methanol.

Activation of strains

In the preparation of the solid culture medium, malt extract (10 g $L^{-1}$), anhydrous dextrose (10 g $L^{-1}$), casein peptone (1 g $L^{-1}$) and bacteriological agar (16 g $L^{-1}$) were used. The Petri dishes (60 mm x 15 mm) and the culture medium were autoclaved (15 min at 1.05 kg $cm^{-2}$). Subsequently, the inoculated plates were preserved for 12 days in the dark at 22.5 ± 2°C.

All strains were inoculated in Petri dishes using 20 g of sterile substrate (1 h at 1.05 kg $cm^{-2}$) at 70% humidity. The *G. lucidum, A. aegerita, P. ostreatus* and *P. djamor* strains were inoculated in Pangola grass; *L. edodes* and *P. eryngii* were inoculated in wood chips, and *A. fuscosuccinea* was inoculated in sorghum grains. The strains were inoculated in two batches; in the first one, an initial metamizole concentration of 50 mg $L^{-1}$ was adjusted, while the second was maintained in the total absence of metamizole sodium. Both batches were incubated in the dark at 22.5 ± 2°C for 28 days.

Degradation of sodium metamizole with crude enzymatic extract

Each one of the strains under study was inoculated in 20 g of their respective substrate. When colonization of the substrate was completed, 50 mL of 0.1 M acetate buffer

solution (pH 4.6) was added. Subsequently, each one of the substrates of the studied strains was macerated, filtered using Whatman No. 2 filter paper, and centrifuged at 2907 G for 10 min. Then, 15 mL of the recovered supernatant (crude enzymatic extract) was taken and poured into a flask (105 mm x 64 mm) together with a solution of metamizole sodium (100 mg $L^{-1}$) to adjust the mixture to a final concentration of 50 mg $L^{-1}$. The flasks were placed on an orbital shaker (Labconco) at 110 rpm at 25 °C for 6 days in the dark.

Effect of temperature and pH on the removal of sodium metamizole

The effects of temperature and pH on the removal of the drug were evaluated for the strain extract that presented the highest degradation (*A. fuscosuccinea).* In this experiment, a factorial design was used ($3^2$), where the first factor, pH, was evaluated at the levels of 4.6, 5.6 and 6.6, and the second factor, temperature, was studied at the levels of 35, 45 and 55°C. Determination of residual metamizole concentration was performed every third day, unless otherwise indicated, using High Pressure Liquid Chromatography (HPLC). The system consisted of a quaternary pump, a 5-channel degasser, and a UV/Vis detector. An isocratic method was used, where the mobile phase consisted of 50% methanol and 50% water. The sample volume injected was 5 µL, and the wavelength was set at 254 nm. The chromatographic column used was Hypersyl® (Agilent, USA) ODS C18 4.0 x 150 mm, with a 5 µm particle diameter. All tests were performed in triplicate. All tests were performed in triplicate. Absorbance readings corresponding to enzymatic activity and protein determination were performed on a UV / Vis spectrophotometer (UV-1700 PharmaSpec Shimadzu).

Enzymatic activity

The laccase activity was determined in a reaction mixture in a buffer solution containing 0.1 M sodium acetate (pH 5.0), 1 mM 2,2'-azino-di (3-ethylbenzthiazoline-6-sulfonate (ABTS) and crude enzymatic extract. The increase in absorbance was monitored at 436 nm ($\varepsilon_{436}$ = 29300 $M^{-1}$ $cm^{-1}$, Rodríguez *et al*. 1999). A reaction mixture containing 0.01% phenol red, 0.1 M sodium succinate (pH 4.5), 100 mM $MnSO_4$, 0.2 mM $H_2O_2$ and crude enzymatic extract was used to determine the manganese peroxidase activity. The reaction was stopped after 10 minutes by adding 5 N NaOH. The increase in absorbance at 610 nm was monitored ($\varepsilon_{610}$ = 22000 $M^{-1}$ $cm^{-1}$, Wariishi *et al*. 1992). The lignin peroxidase activity was measured using a reaction medium consisting of 2 mM $H_2O_2$, 2 mM veratryl alcohol (3,4-dimethoxybenzyl) in 4 M sodium tartrate buffer (pH 3.0) and crude enzymatic extract. The reaction was started by adding hydrogen peroxide. The absorbance change was read at 310 nm ($\varepsilon_{310}$ = 9300 mol $L^{-1}$ $cm^{-1}$, Tien & Kirk 1984).

To determine the phenol oxidase activity, the reaction medium contained 0.1 M catechol prepared in 0.1 M phosphate buffer (pH 7.0) and enzyme extract. The absorbance change was read at 420 nm ($\varepsilon_{420}$ = 3450 mol $L^{-1}$ $cm^{-1}$, Ögel *et al*. 2006). To measure the aryl alcohol oxidase activity, the reaction mixture consisted of 1 mM veratryl alcohol, 50 mM potassium phosphate buffer (pH 6.0) and the crude enzyme extract. The formation of veratraldehyde was read at 310 nm ($\varepsilon_{310}$ = 9300 mol $L^{-1}$ $cm^{-1}$, Okamoto & Yanase 2002). The versatile peroxidase activity was estimated from the formation of $Mn^{+3}$ (Rodríguez *et al*. 2004). The reaction medium contained 0.1 mM $MnSO_4$ prepared in 100 mM sodium tartrate buffer, 0.1 mM $H_2O_2$ and crude enzymatic extract. The absorbance change was read at 238 nm ($\varepsilon_{238}$ = 6500 mol $L^{-1}$ $cm^{-1}$).

Determination of proteins

Total protein determination was performed based on the Bradford method (1976). For this purpose, a calibration curve was prepared using known dilutions of bovine serum albumin (BSA) as the standard. The absorbance reading was performed at 595 nm on a UV/Vis spectrophotometer (UV-1700 Pharmaspec Shimadzu).

Method of drug recovery

To recover the drug, 2 mL of methanol was added to 0.5 mL of a sample taken from the reaction medium (crude enzymatic extract-metamizole). The mixture was vortexed for 1 min and then filtered using Whatman No. 2 filter paper. In this procedure, the recovery rate of sodium metamizole was 100%.

Chromatographic method

The residual concentration of sodium metamizole, as well as that of its degradation metabolites 4-MAA and 4-AA present in the reaction medium, was quantified using HPLC (Perkin Elmer Flexar Quaternary). An isocratic method was used, where the mobile phase consisted of 50% methanol and 50% water. The typical chromatogram for this method is presented in Fig. 1, where the retention times of sodium metamizole (1.032 min), 4-AA (2.601 min) and 4-AAA (3.121 min) are indicated.

Fig. 1

Statistical analysis

The results obtained from degradation tests using crude extract, as well as those obtained in the study of temperature and pH effects on degradation rates, were subjected to analysis of variance (ANOVA) of repeated measures. The results obtained

in enzymatic activity tests were analyzed by one-way ANOVA. All ANOVAs, as well as the Tukey mean separation tests, were performed with a significance level of $\alpha = 0.05$. Each of the bioassays were performed in triplicate, and the SAS statistical software JMP version 4 was used.

## RESULTS AND DISCUSSION

Degradation of sodium metamizole by the crude enzymatic extract of the fungi studied

The results show that in both test groups, only *A. fuscosuccinea* extract degraded metamizole sodium to levels below the limit of detection during the first three days of degradation (Fig. 2). *Pleurotus eryngii* extract achieved a degradation rate of 96% in 6 days, while the decrease in the initial drug concentration remained in the range of 50-75% for the other extracts. A significant interaction between the strains and the time was found (Table. 1), and this was observed in two groups of the tests ($p < 0.001$ in both cases).

We observed that the decrease in the initial concentration of the drug was influenced not only by the strain used but also by the conditions of the substrate (with and without metamizole). This can be attributed to the fact that the enzyme system possessed by each strain differs in the affinity for the substrate or the ability of the fungi to produce one or more enzymes responsible for degradation (Wesenberg *et al*. 2003, Dávila & Vázquez 2006). It is likely that the presence of metamizole in the preculture substrate influenced less production of ligninolytic enzymes. Rodríguez *et al*. (1999) observed that an increase in enzymatic activities in the crude extract of the genera

*Bjerkandera, Pleurotus, Phanerochaete, Sporotrichum* and *Trametes* was positively correlated to their ability to decolorize the extracellular medium. In contrast, Yanez *et al*. (2016) demonstrated a decrease in the laccase and phenoloxidase activities in *A. fuscosuccinea* after 8 days in extracts with endosulfan compared to those that did not contain it.

Fig.2

Table. 1

To study the performance of the crude enzymatic extract from *A. fuscosuccinea*, additional tests were carried out using a degradation time of 6 h. The residual concentration of metamizole was monitored in these tests, as well as the concentration of its two metabolites, 4-MAA and 4-AA (Fig. 3). A control test was also performed to discriminate the reduction of the residual drug concentration due to factors other than those evaluated. In the ANOVA performed on the data obtained in the control tests, the concentration remained unchanged during treatment (d.f. = 4, F = 0.445, p = 0.774).

Tests carried out with advanced oxidation processes, such as homogeneous photocatalysis with photo-Fenton (Pérez *et al*. 2007), photocatalysis with $TiO_2$ (Pérez 2008), Fenton and photo-Fenton (Sekhar & Kumar 2014) and the Electro-Fenton process (Barros *et al*. 2014), have been applied for the degradation of dipyrone and 4-MAA with a degradation rate of approximately 95% in a reaction time of 0.5 h. However, no biological treatments are reported for this molecule or any of its metabolites

generated. Regarding the time of biodegradation for these types of non-steroidal anti-inflammatory drugs (NSAIDs), similar results have been reported using the strain *Trametes versicolor*. Marco *et al*. (2010a) achieved a degradation rate of 94% for diclofenac in the first hour of the reaction, and it was not detected in the liquid medium after 4 h. *Trametes versicolor* is able to biodegrade naproxen by achieving 95% removal in 5 h, and it completely eliminated ketoprofen after 24 h (Marco *et al.* 2010b, Marco *et al*. 2010c).

Fig. 3

Our results show that in the first 15 min, a decrease of more than 20% of metamizole content is observed (Fig. 3) , and 4-MAA and 4-AA are formed as products of that degradation at the same time (Bocca *et al*. 2007, Feldmann *et al*. 2008). Afterward, metamizole decreases steadily for up to 4 h of contact, while the metabolites produced stabilize. The fact that 4-AA does not accumulate further suggests it is probably degraded by the crude extract, while 4-MAA is apparently more resistant to degradation by the extracellular enzymatic system of the fungus because it accumulates up to the $6^{th}$ day (Pérez *et al*. 2007). The concentrations of 4-MAA and 4-AA at the end of the bioassay were 3.81 and 0.43 mg $L^{-1}$, respectively.

Effect of temperature and pH on the degradation of metamizole

The bioassays with the highest rate of degradation in the first 2 h of the reaction followed pseudo-first order kinetics under the following conditions: pH 5.6, 35°C; pH 5.6, 45°C; pH 4.6, 55°C (Fig. 4). Similarly, Machado *et al*. (2013) demonstrated total removal

of the same drug; however, those studies were carried out using electrochemical processes. Ergün *et al*. (2004) reported that although temperature increases the hydrolysis reaction rate of dipyrone to 4-MAA, it is more affected by pH, suggesting acid-catalyzed hydrolysis.

Fig. 4

Protein determination and enzymatic activity of *A. fuscosuccinea*

The protein concentration in the crude extract from *A. fuscosuccinea* at pH 4.6, 5.6 and 6.6 was 0.1037, 0.0756 and 0.1415 mg $mL^{-1}$, respectively. The enzymatic assays showed the presence of laccases, lignin peroxidases and phenol oxidases. The results obtained in the tests performed for both the determination of enzymatic activity and the specific determination showed significant pH influence ($p < 0.0001$). It can be observed that in the case of laccases, the highest enzymatic activity was reached at pH 5.6 and 6.6 (23.47 U $mL^{-1}$), with no significant difference between them, whereas a greater specific activity was observed at pH 5.6 (0.3104 U $mg^{-1}$). For the phenoloxidase enzymes, the highest enzymatic and specific activity were achieved at pH 5.6, which were 11.93 U $mL^{-1}$ and 0.1579 U $mg^{-1}$, respectively (Fig. 5).

Fig. 5

Baldrian (2006) indicates that the majority of laccases exhibit a range of optimum pH values for enzymatic activity in acid, which varies depending on the type of substrate; ABTS requires a pH of 2-5, 2,6-dimethoxyphenol requires a pH between 3-8, and syringaldazine requires a pH between 3.5-7. In constrast, we found that laccase had the

highest activity at pH 5.6, using ABTS as a substrate. For the phenol oxidase enzyme, the highest activity was achieved at pH 5.6. These data agree with those obtained by Yanez *et al.* (2015). On the other hand, Córdova *et al.* (2011) reported that the optimal activity for phenol oxidase from a crude extract of *P. pulmonarius* was achieved in pH ranges between 5 and 6, and Kolcuoğlu (2012) showed optimal pH values between 5 and 7 for a group of purified phenol oxidases.

## CONCLUSION

The enzymatic extracts obtained from the WRF evaluated in this work have been demonstrated to degrade metamizole sodium. The extract obtained from the strain *A. fuscosuccinea* was effective in the total elimination of the drug in a reaction time of 6 h. Monitoring of the 4-MAA and 4-AA metabolites in the first 15 min of the reaction showed that the molecule is rapidly degraded by this extract, with the most favorable conditions being at pH 5.6 at a temperature of 35°C. Also the results of enzymatic activity suggest the probable participation of laccases, phenoloxidases and lignin peroxidases in the degradation process of the molecules under study.

## ACKNOWLEDGEMENTS

The first author wishes to thank the National Council of Science and Technology of Mexico (Conacyt, grant No. 623870) for a scholarship during her Master's Science studies. This research was financially supported by Fondos Mixtos Conacyt by project FOMIX-13149 "Design, construction, equipment and startup of a state center for

innovation and technology transfer for the development of coffee growing in Chiapas, Mexico" and by project MT-11063 of Ecosur "Social and environmental innovation in coffee growing areas for reducing vulnerability". The authors also thank Lilia Moreno Ruiz for her technical help and Javier Valle for his help with statistical analysis.

## REFERENCES

Andreozzi R., Marotta R., Pinto G., Pollio A. (2002) Carbamazepine in water, persistence in the environment, ozonation treatment and preliminary assessment on algal toxicity. *Water Research* **36**(11): 2869–2877.

Baldrian P. (2006) Fungal laccases: occurrence and properties. FEMS *Microbiology Reviews* **30**(2): 215–242.

Barceló L. D., López A. M. J. (2007) Contaminación y calidad química del agua: el problema de los contaminantes emergentes. Fundación Nueva Cultura del Agua. Panel científico-técnico de seguimiento de la política de aguas. Instituto de Investigaciones Químicas y Ambientales-CSIC (Barcelona).

Barros P.W. R., Borges P. M., Reis M. R., Rocha S. R., Bertazzolt R., Lanza V. M. R. (2014) Degradation of dipyrone by the Electro-Fenton Process in an electrochemical flow reactor with a modified gas diffusion electrode. *Journal of the Brazilian Chemical Society*: 1-8 http://dx.doi.org/10.5935/0103-5053.20140160.

Blánquez P., Guieysse B. (2008) Continuous biodegradation of 17β–estradiol y el 17 α-ethynylestradiol by *Trametes versicolor*. *Journal of Hazardous Materials* **150**(2): 459-462.

Bocca C. C., Basso A. E., Mosqueta R. (2007) Conformational study of the four main dipyrone metabolites through theoretical methods: Electrostatic potential maps and NBO calculations. *Journal of Molecular Structure* **815**: 75-81.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* **72**: 248-254.

Córdova J. R. A., Gordillo D. L. L., Bello M. R., Sánchez J. E. (2011) Use of spent substrate after *Pleurotus pulmonarius* cultivation for the treatment of chlorothalonil containing wastewater. *Journal of Enviromental Management* **92**(3): 948-952.

Dávila G., Vázquez D. R. (2006) Enzimas ligninolíticas fúngicas para fines ambientales. *Mensaje Bioquímico* **30**: 29-55.

Ergün H., Frattareli C. D. A., Aranda V. J. (2004) Characterization of the role of physicochemical factors on the hydrolysis of dipyrone. *Journal of Pharmaceutical and Biomedical Analysis* **35**(3): 479-487.

Feldmann D. F., Zuehlke S., Heberer T. (2008) Occurrence, fate and assessment of polar metamizole (dipyrone) residues in hospital and municipal wastewater. *Chemosphere* **71**(9): 1754-1764.

Fent K., Weston A. A., Caminada D. (2006) Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* **76**(2): 122-159.

Gómez P. C. V. (2011) Eliminación de tetraciclinas de las aguas mediante procesos avanzados de oxidación, carbones activados y adsorbentes obtenidos a partir de lodos de depuradora. Tesis doctoral. Universidad de Granada, España.

Gomez M. J., Bueno M. M. J., Lacorte S., Fernandez A. A. R., Aguera A. (2007) Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. *Chemosphere* **66**(6): 993–1002.

He Z., Cheng X., Kysas G., Fu J. (2016) Pharmaceuticals pollution of aquaculture and its management in China. *Journal of Molecular Liquids* **223**: 781-789.

Kartheek R. B., Maheswaran R., Kumar G., Sharmila B. G. (2011) Biodegradation of pharmaceutical wastes using different microbial strains. *International Journal of Pharmaceutical & Biological Archives* **2**(5): 1401-1404.

Kolcuoğlu Y. (2012) Purification and comparative characterization of monophenolase and diphenolase activities from a wild edible mushroom (*Macrolepiota gracilenta*). *Process Biochemistry* **47**(12): 2449-2454.

Kosjek T., Heath E., Kompare B. (2007) Removal of pharmaceutical residues in a pilot wastewater treatment plant. *Analytical and Bioanalytical Chemistry* **387**(4): 1379-1387.

Kümmerer K. (2004) *Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks* 2nd ed Springer-Verlag, Heidelberg, 527 p.

Lloret L., Eibes G., Lú Chau T. A., Moreira M. T., Feijoo G., Lema J. M. (2010) Laccase-catalyzed degradation of anti-inflamatories and estrogens. *Biochemical Engineering Journal* **51**: 124-131.

Machado R. R., Fragale B. J. A., Lanzoni M. F., da Silva R. R., Ribeiro B. M., Gomes F. N., de Vasconcelos L. M. R. (2013) Degradation of dipyrone in an electrochemical flow-by reactor using anodes of boron-doped diamond (BDD) supported on titanium. *Journal of Electroanalytical Chemistry* **690**: 89-95.

Marco U. E., Pérez T. M., Cruz M. C., Caminal G., Vicent T. (2010a) Degradation of the drug sodium diclofenac by *Trametes versicolor* pellets and identification of some intermediates by NMR. *Journal of Hazardous Materials* **176**: 836-842.

Marco E., Pérez T. M., Blánquez P., Vicent T., Caminal G. (2010b) Biodegradation of the analgesic naproxen by *Trametes versicolor* and identification of intermediates using HPLC-DAD-MS and NMR. *Bioresource Technology* **101**(7): 2159-2166.

Marco U. E., Pérez M., Cruz M. C., Caminal G., Vicent T. (2010c) White rot fungus-mediated degradation of the analgesic ketoprofen and identification of intermediates by HPLC-DAD-MS and NMR. *Chemosphere* **78**(4): 474-481.

Martínez B. M. J., Agüera A., Gómez M. J., Hernando M. D., García R. J. F., Fernández A. A. R. (2007) Application of liquid chromatography/quadrupole-linear ion trap mass spectrometry and time-offlight mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater. *Analytical Chemistry* **79**(24): 9372-9384.

Miceli M. A. S., Nájera A. H. A. Rojas V. M. N., Quintero L. L. I., Orantes G. C. (2014) Revisión de tecnologías para el tratamiento de aguas residuales incluyendo algunos fármacos. *Innovación más Desarrollo* **3**(5): 73-95.

Nash J. P., Kime D. E., Van der Ven T. M., Wester P. W., Brion F., Maack G., Stahlschmidt A. P., Tyler C. R. (2004) Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. *Environmental Health Perspectives* **112**(17): 1725-1733.

Ögel Z. B., Yüzügüllü Y., Mete S., Bakir U., Kaptan Y., Sutay D., Demir A.S. (2006) Production, properties and application to biocatalysis of a novel extracellular alkaline

phenol oxidase from the thermophilic fungus *Scytalidium thermophilum*. *Applied Microbiology and Biotechnology* **71**(6): 853–862.

Okamoto K., Yanase H. (2002) Aryl alcohol oxidase from the white-rot basidiomycete *Pleurotus ostreatus*. *Mycosciencie* **43**: 391-395.

Pérez E. L. A. (2008) Degradación fotoquímica de contaminantes emergentes mediante procesos solares. Evaluación analítica. Tesis doctoral. Universidad de Almería, España.

Pérez E. L. A., Malato S., Agüera A., Fernández A. A. R. (2007) Degradation of dipyrone and its main intermediates by solar AOPs identification of intermediate products and toxicity assessment. *Catalysis Today* **129**: 207-214.

Pointing S. B. (2001) Feasibility of bioremediation by white-rot fungi. *Applied Microbiology Biotechnology* **57**: 20-33.

Quesada P. I., Jáuregui H. U. J., Wilhelm A. M., Delmas H. (2009) Contaminación de las aguas con productos farmacéuticos. Estrategias para enfrentar la problemática. *CENIC Ciencias Biológicas* **40** (3): 173-179.

Quintero D. J. C. (2011) Revisión: Degradación de plaguicidas mediante hongos de la pudrición blanca de la madera. *Revista Facultad Nacional de Agronomía-Medellín* **64**(1): 5867-5882.

Robles H. L., González F. A. C., Crawford L. D., Chun C. W. W. (2008) Review of enviromental organopollutants degradation by white-rot basidiomycete mushrooms. *Tecnociencia Chihuahua* **2**(1): 32-40.

Rodarte M. A. I., Moreira M. T., Feijoo G., Lema J. M. (2010) Evaluation of two fungal strains for the degradation of pharmaceutical and personal care products (PPCPs). *Chemical Engineering Transactions* **20**: 31-36.

Rodríguez E., Pickard A. M., Vázquez D. R. (1999) Industrial dye decolorization by laccases from ligninolytic fungi. *Current Microbiology* **30**: 27-32.

Rodríguez E., Nuero O., Guillén F., Martínez A. T., Martínez M. J. (2004) Degradation of phenolic and non-phenolic aromatic pollutants by four *Pleurotus* species: the role of laccase and versatile peroxidase. Soil Biology and Biochemistry **36**: 909-916.

Ruiz A. G. M. L., Fernández S. J. M., Rodríguez V. R., Poggi V. H. (2002) Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil. *Advances in Enviromental Research* **6**(4): 559-568.

Santos I. J. S., Grossman J. M., Sartoratto A., Ponezi A., Durrant L. (2012) Degradation of the recalcitrant pharmaceuticals carbamazepine and 17$\alpha$-ethinylestradiol by ligninolytic fungi. *Chemical engineering transactions* **27**: 169-174.

Schäfers C., Teigeler M., Wenzel A., Maack G., Fenske M., Segner H. (2007) Concentration and time dependent effects of the synthetic estrogen, 17$\alpha$-ethinylestradiol, on reproductive capabilities of the zebrafish, Danio rerio. *Journal of Toxicology and Environmental Health* **70**(9): 768-79.

Sekhar G. A., Kumar G. A. (2014) Fenton, Photo-Fenton, $H_2O_2$ Photolysis, and $TiO_2$ Photocatalysis for dipyrone oxidation: drug removal, mineralization, biodegradability, and degradation mechanism. *Industrial and Engineering Chemistry Research* **53**: 1351-1358.

Szabó Z., Szoboszlai N., Jámbor E., Gulyás G., Lóránd T., Ohmacht R., Záray G., Mihucz V. G. (2013) Determination of four dipyrone metabolites in Hungarian municipal wastewater by liquid chromatography mass spectrometry. *Microchemical Journal* **107**: 152-157.

Ternes T., Giger W., Joss A. (2006) Introduction. In: Ternes T, Joss A (eds) *Human pharmaceuticals, hormones and fragrances: the challenge of micro pollutans in urban water management*. IWA Publishing, London, pp 1-5. ISBN 1843390930.

Tien M., Kirk T. K. (1984) Lignin degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization and catalytic properties of unique $H_2O_2$–requiring oxygenase. *Proceedings of the National Academy of Sciences* **81**(8): 2280–2284.

Wariishi H., Valli K., Gold M. H. (1992) Maganese (II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*. *The Journal of Biological Chemistry* **267**(33): 23688–23695.

Watanabe R., Sales P., Campos L., García T., Valadares M., Schimidt F., Santiago M. (2012) Evaluation of the use of *Pycnoporus sanguineus* fungus for phenolics and genotoxicity decay of a pharmaceutical effluent treatment. *Ambi-Agua* **7**(3): 41-50.

Wesenberg D., Kyriakides I., Agathos S. N. (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnology Advances* **22**: 161–187.

Yanez M. A. F., Sánchez J. E., Vázquez D. R., Cruz L. L., Calixto R. M. A. (2015) Purification and partial characterization of a phenol oxidase from the edible mushroom *Auricularia fuscosuccinea. Jacobs Journal of Enzymology and Enzyme Engineering* **1**(1): 1-6

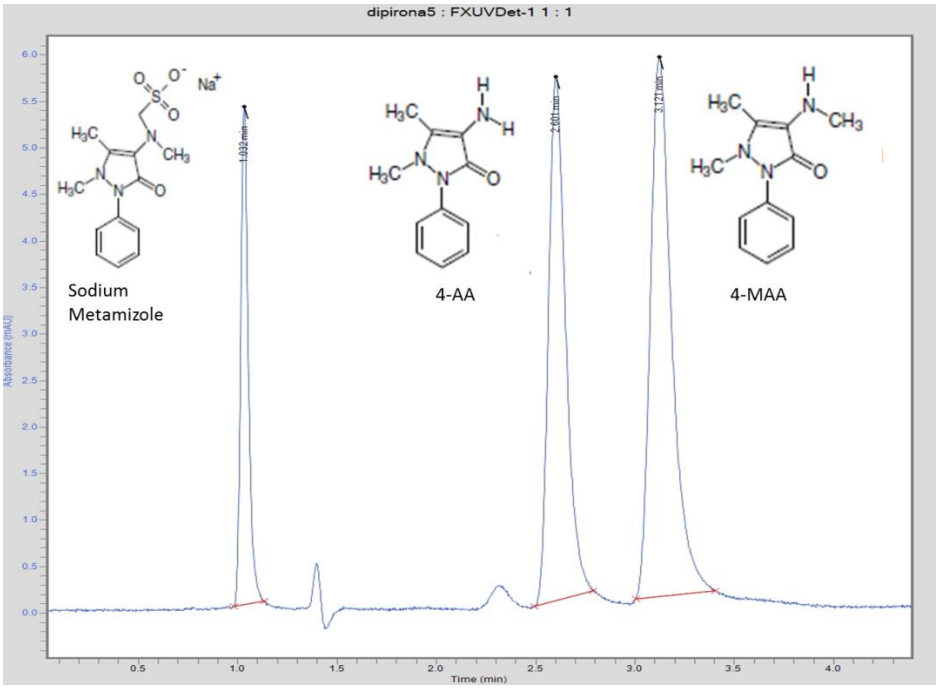
Yanez M. A. F., Sánchez J. E., Vázquez D. R., Cruz L. L., Calixto R. M. A. (2016) Degradation of endosulfan by strains of *Auricularia fuscosuccinea*. *Sydowia* **68**: 7-15.

Yongjun Z., Sven-Uwe G., Gal G. (2008) Carbamazepine and diclofenac: Removal in wastewater treatment. *Chemosphere* **73**(8): 1151–1161.

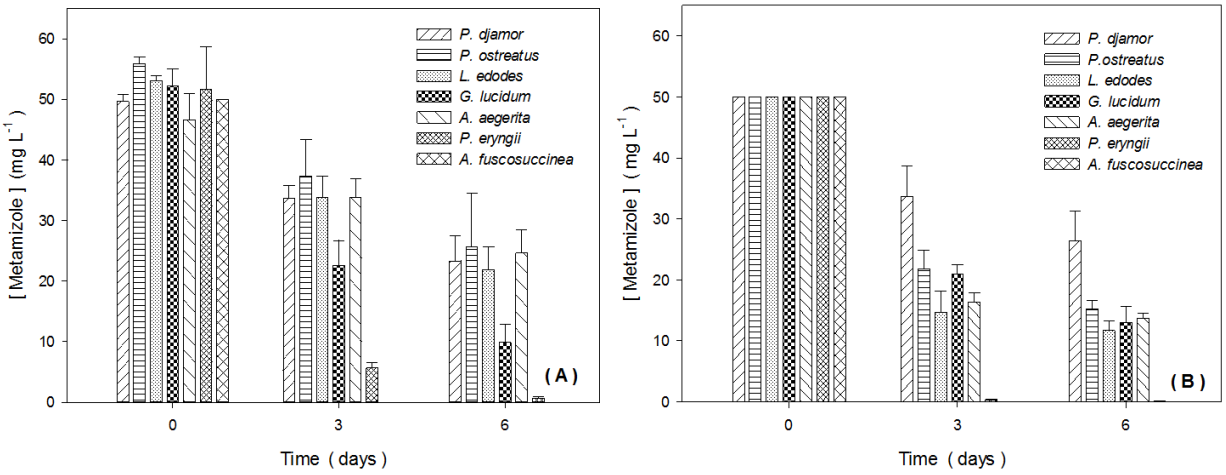
**Table.1.-** ANOVA of repeated measures of degradation test data using crude enzyme extract

| | (a) ANOVA SCPM* | | | | (b) ANOVA SCWM** | | | |
|---|---|---|---|---|---|---|---|---|
| | **NumDF** | **DenDF** | **F** | **p** | **NumDF** | **DenDF** | **F** | **P** |
| **Strain** | 6 | 14 | 25.54 | <0.0001 | 6 | 14 | 108.12 | <0.0001 |
| **Time** | 2 | 28 | 1097.07 | <0.0001 | 2 | 28 | 2338.00 | <0.0001 |
| **Strain:Time** | 12 | 28 | 27.89 | <0.0001 | 12 | 28 | 29.82 | <0.0001 |

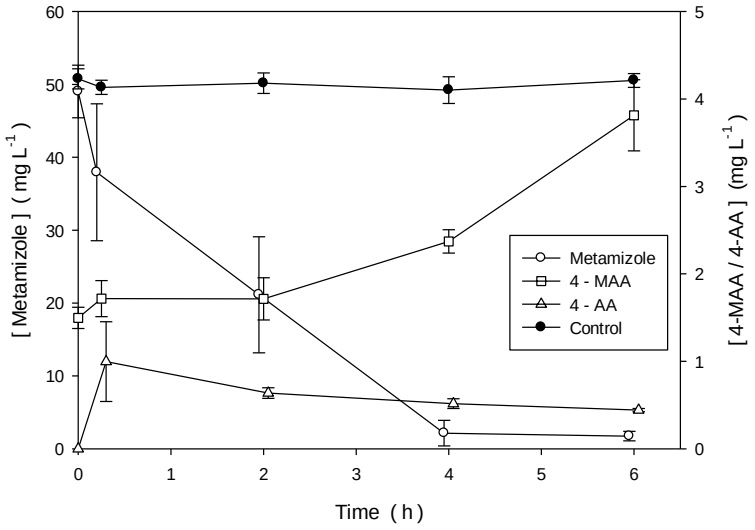
* SCPM: substrate colonized in presence of metamizole. **SCWM: Substrate colonized without metamizole. NumDF: degrees of freedom of numerador. DenDF: degrees of freedom of error.


dipirona5 : FXUVDet-1 1 : 1
Sodium
Metamizole
4-AA
4-MAA
Time (min)

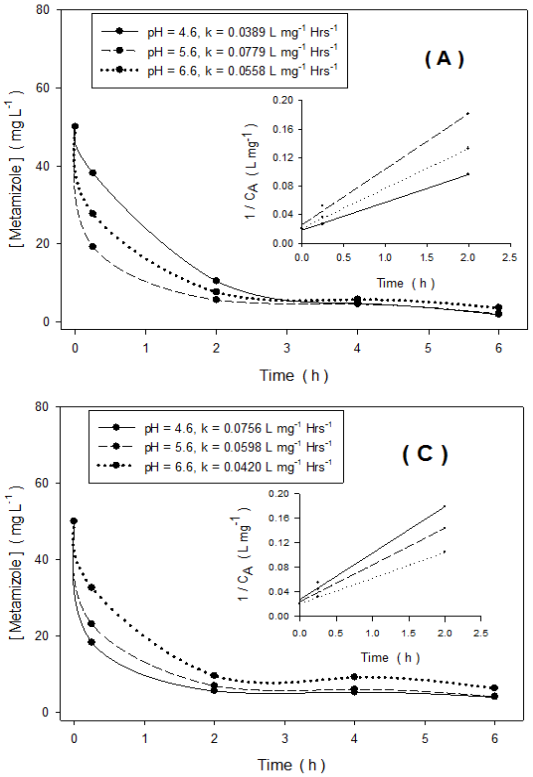
**1**


[ Metamizole ] (mg L-1)
Time ( days )
P. djamor
P. ostreatus
L. edodes
G. lucidum
A. aegerita
P. eryngii
A. fuscosuccinea
( A )
P.ostreatus
( B )

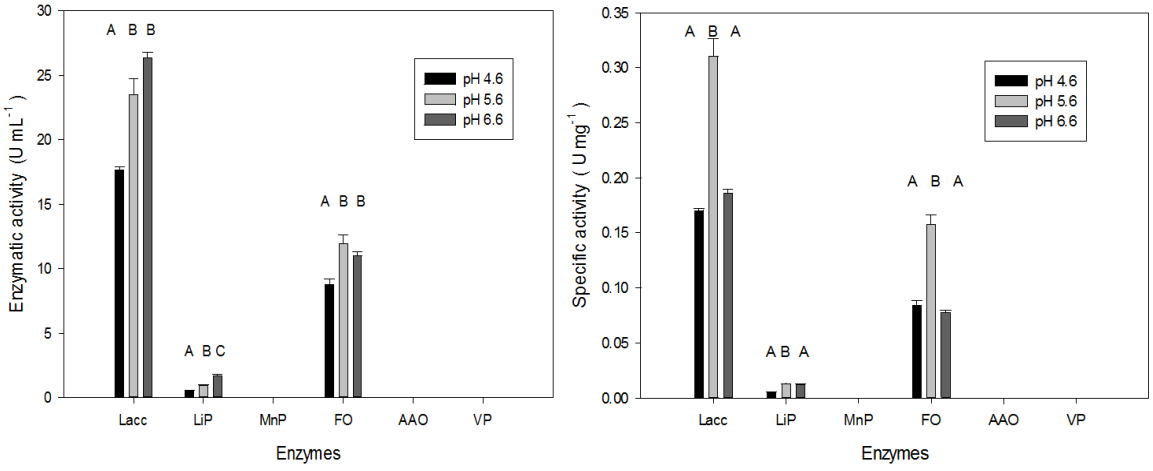
**2**


[ Metamizole ] ( mg L -1 )
[ 4-MAA / 4-AA ] (mg L -1 )
Time ( h )
Metamizole
4 - MAA
4 - AA
Control
0
10
20
30
40
50
60
0
1
2
3
4
5
0
1
2
3
4
5
6

**3**


pH = 4.6, k = 0.0389 L mg-1 Hrs-1
pH = 5.6, k = 0.0779 L mg-1 Hrs-1
pH = 6.6, k = 0.0558 L mg-1 Hrs-1
( A )
[ Metamizole ] ( mg L-1 )
Time ( h )
1 / CA ( L mg-1 )
pH = 4.6, k = 0.0531 L mg-1 Hrs-1
pH = 5.6, k = 0.0638 L mg-1 Hrs-1
pH = 6.6, k = 0.0424 L mg-1 Hrs-1
( B )
pH = 4.6, k = 0.0756 L mg-1 Hrs-1
pH = 5.6, k = 0.0598 L mg-1 Hrs-1
pH = 6.6, k = 0.0420 L mg-1 Hrs-1
( C )

**4**


Enzymatic activity (U mL-1 )
Specific activity ( U mg-1 )
pH 4.6
pH 5.6
pH 6.6
A B B
A B C
A B B
A B A
A B A
Lacc
LiP
MnP
FO
AAO
VP
Enzymes

**5**

## Figure legends

**Fig. 1**. Chromatogram obtained from HPLC showing the presence of metamizole and two of its metabolites; metamizole sodium (RT 1.032 min), 4-aminoantipyrine (RT 2.601 min) and 4-methylaminoantipyrine (RT 3.121 min).

**Fig. 2**. Degradation profile of sodium metamizole using the enzyme extract from strains previously cultured in the presence of the drug (A) and without the drug (B). Bioassay conditions: pH = 4.6, T = 25°C, agitation = 110 rpm, time = 6 days in the dark.

**Fig. 3**. Degradation profile of sodium metamizole and generation of its metabolites by an aqueous enzymatic extract from *A. fuscosuccinea* ECS-0210. Bioassay conditions: pH = 4.6, T = 25°C, agitation = 110 rpm, time = 6 hours in the dark.

**Fig. 4.** Effect of pH and temperature on the degradation of sodium metamizole by an enzymatic extract of *A. fuscosuccinea*. Box: Degradation rate. Bioassay conditions: agitation = 110 rpm, time = 6 h in the dark, pH (4.6, 5.6, 6.6), temperature A) 35°C, B) 45°C and C) 55°C

**Fig. 5.** Enzymatic activity and specific activity in the crude extract from *A. fuscosuccinea* under varying pH conditions. Laccase (Lacc), Lignin peroxidase (LiP), Manganese peroxidase (MnP), Phenol oxidase (FO), Aryl alcohol oxidase (AAO) and Versatile peroxidase (VP). The homogeneous groups generated with the Tukey mean separation test are presented in capital letters.

## III. Conclusiones

1. Los extractos enzimáticos obtenidos a partir de *Auricularia fuscosuccinea, Lentinula edodes, Ganoderma lucidum, Agrocybe aegerita, Pleurotus ostreatus, P. djamor* y *P. eryngii* evaluadas en este trabajo demostraron su capacidad para degradar el metamizol sódico.
2. Los extractos enzimáticos provenientes de cultivos de hongos en ausencia del fármaco mostraron mayores porcentajes de degradación.
3. El extracto obtenido con la cepa *A.fuscosuccinea* resultó ser el más eficaz en la eliminación total del fármaco en un tiempo de reacción de 6 horas.
4. El monitoreo de los metabolitos 4-MAA y 4-AA en los primeros 15 minutos de reacción mostró que la molécula es degradada rápidamente por el extracto obtenido con la cepa *A.fuscosuccinea*, siendo las condiciones más favorables a pH 5.6 y 35 °C.
5. La mayor actividad enzimática y específica de las enzimas lacasa y fenoloxidasa se presentó a pH 5.6.
6. Los ensayos de actividad enzimática demostraron la expresión de actividad de enzimas lacasas, fenoloxidasas y lignino peroxidasas en el proceso de degradación del metamizol sódico. Posteriores estudios de degradación del fármaco con las enzimas puras podrían indicar si ellas participan o no en la degradación.

## IV. Literatura citada


Andreozzi, R., Marotta, R., Pinto, G., Pollio, A. 2002. Carbamazepine in water, persistence in the environment, ozonation treatment and preliminary assessment on algal toxicity. *Water Research* **36**(11): 2869–2877.

Arrieta, P. R., Guerra, N. M., 2012. Modelado de un reactor fotocatalítico heterogéneo solar con $TiO_2$ para el tratamiento de efluentes contaminados con residuos farmacéuticos. Tesis de licenciatura. Universidad de Cartagena, Colombia.

Barceló, L. D., López A. M. J., 2007. Contaminación y calidad química del agua: el problema de los contaminantes emergentes. Fundación Nueva Cultura del Agua. Panel científico- técnico de seguimiento de la política de aguas. Instituto de Investigaciones Químicas y Ambientales-CSIC (Barcelona).

Barros, P.W. R., Borges, P. M., Reis, M. R., Rocha, S. R., Bertazzolt, R., Lanza, V. M. R., 2014. Degradation of dipyrone by the Electro-Fenton Process in an electrochemical flow reactor with a modified gas diffusion electrode. *Journal of the Brazilian Chemical Society*: 1-8 http://dx.doi.org/10.5935/0103-5053.20140160.

Blánquez, P., Guieysse B., 2008. Continuos biodegradation of 17$\beta$–estradiol y el 17 $\alpha$-ethynylestradiol by *Trametes versicolor*. *Journal of Hazardous Materials* **150**(2): 459-462.

Chong, N., Jin, B., Chow C., Saint, C. (2010). Review. Recent developments in photocatalytic water treatment technology: A review. *Water Research* **44**: 2997-3027.

Ergün, H., Frattareli, C. D. A., Aranda, V. J., 2004. Characterization of the role of physicochemical factors on the hydrolysis of dipyrone. *Journal of Pharmaceutical and Biomedical Analysis* **35**(3): 479-487.

Feldmann, D. F., Zuehlke, S., Heberer T., 2008 Occurrence, fate and assessment of polar metamizole (dipyrone) residues in hospital and municipal wastewater. *Chemosphere* **71**(9): 1754-1764.

Fent, K., Weston, A. A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* **76**(2): 122-159.

Gómez, P. C. V., 2011. Eliminación de tetraciclinas de las aguas mediante procesos avanzados de oxidación, carbones activados y adsorbentes obtenidos a partir de lodos de depuradora. Tesis doctoral. Universidad de Granada, España.

Gomez, M. J., Bueno, M. M. J., Lacorte, S., Fernandez, A. A. R., Aguera, A., 2007. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. *Chemosphere* **66**(6): 993–1002.

He, Z., Cheng, X., Kysas, G., Fu, J., 2016. Pharmaceuticals pollution of aquaculture and its management in China. *Journal of Molecular Liquids* **223**: 781-789.

Kartheek, R. B., Maheswaran, R., Kumar, G., Sharmila, B. G., 2011. Biodegradation of pharmaceutical wastes using different microbial strains. *International Journal of Pharmaceutical & Biological Archives* **2**(5): 1401-1404.

Kosjek, T., Heath, E., Kompare, B., 2007. Removal of pharmaceutical residues in a pilot wastewater treatment plant. *Analytical and Bioanalytical Chemistry* **387**(4): 1379-1387.

Kümmerer, K., 2004. *Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks* 2nd ed Springer-Verlag, Heidelberg, 527 p.

Lloret, L., Eibes, G., Lú Chau T. A., Moreira, M. T., Feijoo, G., Lema, J. M., 2010. Laccase-catalyzed degradation of anti-inflamatories and estrogens. *Biochemical Engineering Journal* **51**: 124-131.

Marco, U. E., Pérez, T. M., Cruz, M. C., Caminal, G., Vicent, T., 2010a. Degradation of the drug sodium diclofenac by *Trametes versicolor* pellets and identification of some intermediates by NMR. *Journal of Hazardous Materials* **176**: 836-842.

Marco, E., Pérez, T. M., Blánquez, P., Vicent, T., Caminal, G., 2010b. Biodegradation of the analgesic naproxen by *Trametes versicolor* and identification of intermediates using HPLC-DAD-MS and NMR. *Bioresource Technology* **101**(7): 2159-2166.

Martínez, B. M. J., Agüera, A., Gómez, M. J., Hernando, M. D., García, R. J. F., Fernández, A. A. R., 2007. Application of liquid chromatography/quadrupole-linear ion trap mass spectrometry and time-offlight mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater. *Analytical Chemistry* **79**(24): 9372-9384.

Martínez, M. J., Hernando, M., Herrera, S., Gómez, M., Fernández A., Bustamante I., García, E., 2010. Pilot survey of chemical contaminants from industrial and human activities in river water of Spain. *International Journal Environmental Analytical Chemistry* **90**: 321-343.

Miceli, M. A. S., Nájera, A. H. A., Rojas, V. M. N., Quintero, L. L. I., Orantes, G. C. 2014. Revisión de tecnologías para el tratamiento de aguas residuales incluyendo algunos fármacos. *Innovación más Desarrollo* **3**(5): 73-95.

Nash, J. P., Kime, D. E., Van der Ven, T. M., Wester, P. W., Brion, F., Maack, G., Stahlschmidt, A. P., Tyler, C. R., 2004. Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. *Environmental Health Perspectives* **112**(17): 1725-1733.

Nikolaou, A., Meric, S., Fatta D., 2007. Occurrence patterns of pharmaceuticals in water and wastewater environments. Annal Bioanal Chem. 387: 1225-1234.

Pérez, E. L. A., 2008. Degradación fotoquímica de contaminantes emergentes mediante procesos solares. Evaluación analítica. Tesis doctoral. Universidad de Almería, España.

Pointing, S. B., 2001. Feasibility of bioremediation by white-rot fungi. *Applied Microbiology Biotechnology* **57**: 20-33.

Quesada, P. I., Jáuregui, H. U. J., Wilhelm, A. M., Delmas, H., 2009. Contaminación de las aguas con productos farmacéuticos. Estrategias para enfrentar la problemática. *CENIC Ciencias Biológicas* **40** (3): 173-179.

Quintero, D. J. C. 2011. Revisión: Degradación de plaguicidas mediante hongos de la pudrición blanca de la madera. *Revista Facultad Nacional de Agronomía-Medellín* **64**(1): 5867-5882.

Robinson, T., McMullan, G., Marchant, R., Nigam. P.,2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresource Technology* **77** (3): 247-255.

Robles, H. L., González, F. A. C., Crawford, L. D., Chun, C. W. W. 2008. Review of enviromental organopollutants degradation by white-rot basidiomycete mushrooms. *Tecnociencia Chihuahua* **2**(1): 32-40.

Rodarte, M. A. I., Moreira, M. T., Feijoo, G., Lema, J. M., 2010. Evaluation of two fungal strains for the degradation of pharmaceutical and personal care products (PPCPs). *Chemical Engineering Transactions* **20**: 31-36.

Rubilar, A. O., 2007. Biorremediación de suelos contaminados con pentaclofenol (PCF) por hongos de pudrición blanca. Tesis doctoral. Universidad de la Frontera, Temuco, Chile.

Ruiz, A. G. M. L., Fernández, S. J. M., Rodríguez, V. R., Poggi, V. H., 2002. Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil. *Advances in Enviromental Research* **6**(4): 559-568.

Santos, I. J. S., Grossman, J. M., Sartoratto, A., Ponezi, A., Durrant, L., 2012. Degradation of the recalcitrant pharmaceuticals carbamazepine and 17α-ethinylestradiol by ligninolytic fungi. *Chemical engineering transactions* **27**: 169-174.

Schäfers, C., Teigeler, M., Wenzel, A., Maack, G., Fenske, M., Segner, H., 2007. Concentration and time dependent effects of the synthetic estrogen, 17α-ethinylestradiol, on reproductive capabilities of the zebrafish, Danio rerio. *Journal of Toxicology and Environmental Health* **70**(9): 768-79.

Stuart, M., Lapworth D., Crane, S., Muller, S., 2012. Review of risk from potential emerging contaminants in UK groundwater. *Science of the Total Enviroment* **416**: 1-21

Szabó, Z., Szoboszlai, N., Jámbor, E., Gulyás, G., Lóránd, T., Ohmacht, R., Záray, G., Mihucz, V. G., 2013. Determination of four dipyrone metabolites in Hungarian municipal wastewater by liquid chromatography mass spectrometry. *Microchemical Journal* **107**: 152-157.

Ternes, T., Giger, W., Joss, A., 2006. Introduction. In: Ternes T, Joss A (eds) *Human pharmaceuticals, hormones and fragrances: the challenge of micro pollutans in urban water management*. IWA Publishing, London, pp 1-5. ISBN 1843390930.

Watanabe, R., Sales, P., Campos, L., García, T., Valadares, M., Schimidt, F., Santiago, M., 2012. Evaluation of the use of *Pycnoporus sanguineus* fungus for phenolics and genotoxicity decay of a pharmaceutical effluent treatment. *Ambi-Agua* **7**(3): 41-50.

Yongjun, Z., Sven-Uwe, G., Gal, G., 2008. Carbamazepine and diclofenac: Removal in wastewater treatment. *Chemosphere* **73**(8): 1151–1161.