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Full Length Research Paper

Phytochemical analysis and antinociceptive activity of bitter ginger (*Zingiber zerumbet*) cultivated in Manaus/Amazonas

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The present work concerns the analgesic effects of zerumbona, obtained from *Zingiber zerumbet* L. Smith cultivated in Manaus/Amazonas. The compound has been studied for decades because it has potent cytotoxic activity against liver and prostate tumor cells, colon, and breast. The plant is rich in sesquiterpenes, glycosylated flavonoids that present important pharmacological activities; standing out cytotoxic activity against neoplastic cells of cancers. The objective of this study was to test the antinociceptive activity of zerumbone (ZER) using chemical and thermal nociception models, that is, writhing test induced by acetic acid and hot plate test. ZER administered orally and intraperitoneally produced significant and dose-dependent analgesic activity against the pain, acetic acid, formalin, and capsaicin models. In addition, ZER significantly increased the dormancy of the animals in the hotplate test pain model (49-540C). It was demonstrated that intraperitoneal (i.p.) and oral (p.o.) administration of ZER sesquiterpene in doses of 150 to 500 mg/kg i.p. and 250 to 1500 mg/kg p.o. produced significant dose-dependent inhibition of acetic acid-induced abdominal writhing, as compared to fentanest (20 µg/kg). At the same intraperitoneally and orally doses, the ZER produced significant dose-dependent latency-time increases in the hot-plate test relative to control. It was concluded that ZER exhibits both central and peripheral antinociceptive activity, indicating it to hold therapeutic potential for the discovery of new antinociceptive drugs as an alternative for the discovery of new drugs in the control of neurogenesi. The ZER exhibited similar efficacy and strength via both oral and intraperitoneally routes. ZER is the major essential oil component, a new sesquiterpene responsible for its nociceptive effect, when compared with other antinociceptive sesquiterpenes described in literature.

Key words: *Zingiber zerumbet*, zerumbone, Zingiberacea, antinociceptive activity.

INTRODUCTION

Zerumbone (ZER) is the main component extracted from the essential oils of *Zingiber zerumbet*, a plant belonging to family Zingiberaceae, which is much used in the Malaysian traditional medicine (Dev, 1960; Koshimizu et al., 1988). However, in Amazonia, it is only used as an ornamental plant with no therapeutical ends, being

popularly known as bitter ginger. The results obtained with the essential oils extracted by steam distillation provided the lowest, yet very pure, yield percentage (99.95%) purer than that of the previous method (97%), postulated in the patent number PI0505343 - 9/28/11/2007. Dichlorometahne (DCM), methanol (MEOH) and ZER extracts phytochemical screening findings revealed the presence of the following compounds: terpenoids, xanthones, flavonoids, phenols, tannins and alkaloids. EM and DCM TLC indicated a stain revealed in iodine and ceric sulfate with the retention factor of approximately 0.7 cm similar to that of Zerumbona (0.8) and MEOH showing stains in iodine and UV 254 nm. Of all parts of the plant, the RZZ has been the subject of extensive chemical investigations because of its high medicinal values. Various reports have been published regarding the phytochemical content of RZZ. Attempts to isolate and identify bioactive compounds from the RZZ started since 1944 with the identification of humulene (Eddy and Leimback, 1987); monoterpenes (Carter, 1991; Hwang and Wilcox, 1987), and zerumbone (2,6,10-cy-cloundecatrien-1-one, 2,6,9,9-tetramethyl-, (E,E,E)-) (Carter, 1991) from the essential oil of RZZ (EOZZ). It is a substance, presenting antiinflamatory (Murakami et al., 2002), antiproliferative (Takada et al., 2005), antimicrobial (Abdul et al., 2008), antibacterial (Kitayama et al., 2001) and antitumoral (Murakami et al., 2004) activities, being recommended for the treatment of liver, colon and skin cancer (Sulamain et al., 2009). Zerumbone is a monocyclic sesquiterpene compound isolated from rhizomes of *Z. zerumbet*. Recently some scientific research on the bioactivities of zerumbone, which was identified as a major compound of *Z. zerumbet*, reported that zerumbone possesses many pharmacological activities, such as chemoprevention Yob et al. (2011), antiinflammatory and antiallergic activities. Somchit et al. (2005) have earlier reported on the antinociceptive profiles of AEZZ and EEZZ administered intraperitoneally into rats and assessed using the 0.6% acetic acid-induced writhing test.

ZER presents high pharmacological potential, holding great relevance in the pharmaceutical industry application, and it can be utilized in natural medicine manufacturing and cosmetics formulation (Koshimizu et al., 1988). Recent studies using the rhizome essential oil of *Z. zerumbet* showed it to possess both central and peripheral antinociceptive activity when tested using chemical and thermal models of nociception (Takada et al., 2005) and to produce significant antinociceptive effect in all nociception models in mice when given via intraperitoneally route, presenting antinociceptive effect in the peripheral region (Perimal et al., 2011).

In another recent study, ZER produced its antinociception through the activation of nitric oxide (NO)/cyclic guanosine monophosphate (cGMP)/protein kinase C (PKC)/K+ channel pathways (Lapa et al., 2003). However, it is believed that *Z. zerumbet* essential oils antinociceptive activity is linked to its main component, the sesquiterpene ZER. The present work verified this believe using animal models, thereby revealing its potential in the search for new analgesic drugs.

MATERIALS AND METHODS

Plant

The rhizome of *Z. zerumbet* (Zingiberaceae) was collected in the Tarumã, and two exsicats were forwarded to the herbarium at INPA for botanical determination. They were identified by Prof. Dr. Paul Maas (Departament of Plant Ecology and Evolucionary Biology) - herbarium at the University of Utrecht. A voucher specimen (no. 186913) was deposited at the Herbarium of the INPA.

Essential oil extraction and compound isolation

Attaining ZER from *Z. zerumbet* rhizomes essential oils was accomplished utilizing a steam drag distillator. Twenty of fresh and dried rhizomes were weighed on a digital scale, then ground in an electric grinder followed by hand grater. The ground rhizome was placed in a 12 L Mariott flask, coupled to a 20 L semi industrial pressure pot, and 15 L of water or more were poured into it. Temperature control (100°C) and pressure in essential oils destilation was recorded in a Tecnal digital water circulator connected to a condensator (temperature and pressure gauges coupled to a manometer adapted to the pot's lid) linked to the Clevenger device. The graduated sorter was utilized for collecting and verifying the yields of the essential oils. The initial temperature of distilation by vaporisation was 80°C, being later reduced to 70°C and the condensation temperature was 5 to 150°C. Extraction took 4 h. The obtained essential oils were submitted to re-crystallization. The ZER was otained through the national patent deposit process number PI0505343-9 with a purity grade of 97%.

Chemical analyses

Thin layer chromatography analyses (TLC) were conducted on a Merck sílica-gel G 60 plate and developed with iodine vapors, ultraviolet radiation (254 and 366 nm), ceric sulphate and Dragendorff reagent.The chromatographic profile of the samples was also determined through sorting analytical technique by High Efficiency Liquid Chromatography (HELC) in liquid solid stationary phase. The chromatograms were analyzed using a Shimadzu chromatograph, model QP010, column µ-Bondapack CN, 100×8 mm having as mobile phase in water:metanol (80:20) at 6 mL/min and detected at 254 nm**.** The Hydrogen Nuclear Magnetic Ressonance spectra (1H NMR) and Carbon thirteen Nuclear Magnetic Ressonance (13C NMR were carried out at the Biotechnology Center of Amazonia). 1H NMR analyses spectra were recorded in Varian - Mercury 500 MHz sptectrometer with samples dissolved in CDCl3 (deuterared chloroform) and

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CD3OD.D2O (monohydrated methanol). The 13C NMR spectra were registered in Brüker mod. AC - 200 spectrometers operating at 50.3 MHz, the CDCl3 was the solvent utilized and TMS was the inner standard utilized in both resonances. The infrared region (IR) spectra were performed in Perkin-Elmer 1420 spectrometer using potassium bromide (KBr) tablets. Mass spectra (MS) were registered in Finnigan, 4020 model spectrometers.

Animals

For the use of animals *in vivo*, this project was submitted to the Committee on Ethics and Use of Animals (CEUA)/INPA, being approved by the number of opinion 005/2012, in accordance with the current norms. The animals utilized in the experiments were albino mice (*Mus musculus* - albinus variety) weighing between 18 and 30 g and albino rats (Ratus norvegicus - Wistar variety) (male and female) with weight ranging from 150 and 300 g acquired from the Amazon Research Institute Bioterium.

Drugs and chemicals

The following drugs were used: fentanest, dipirone, indometacine, diclorometane (DCM) and methanol (MeOH), acetic acid (Sigma Chemical), and tween 20% (Sigma Chemical). All drugs were dissolved in 0.9% saline solution. The ZER (LTQPN/COTI/INPA) was dissolved in 1% (v/v) Tween 20. Respective controls received only 1% Tween 20% as a vehicle. All drugs, chemicals and ZER solutions were prepared just prior to experiments, administer orally, and intraperoneally route to mice.

Experiments

Acetic acid-induced abdominal writhing

The procedure used was similar to the one described earlier (Koster et al., 1959). Mice were pretreated orally with ZER (50, 250, 500, 1000, 1500 mg/kg, p.o.), 1 h prior to i.p. injection of 1% acetic acid (v/v). The control group received a similar volume of vehicle (0.01 mL/100 g; i.p). Fentanest (20 µg/kg s.c.), was used as the reference drug and administered 30 min before the nociceptive agent. Following the i.p. injection of acetic acid, the animals were immediately placed into a perspex chamber and the number of writhings was recorded for 15 to 120 min, starting from 10 min post injection, as test standards (Rocha and Silva, 1968).

Hot plate

The hot plate test was performed to assess the central antinociceptive properties of ZER according to the method described previously (Koster et al., 1959) with minor modifications. In this test, the hot plate (HOT PLATE F361-INSIGHT) was maintained at 50°C. Animals were placed in the perspex cylinder on the heated surface, and the latency to a discomfort reaction (licking paws or jumping) was determined before and after ZER or drug administration. The ZER (250, 500, 1000, 1500 mg/kg, p.o. and 150, 200, 220, 250, 500 mg/kg, p.i.), vehicle (saline 0.9%; tween 20%) and fentanest (20 µg/kg s.c.) were administered 30 min prior to the beginning of the experiment. Animals were observed before and 30, 60, 120, 180 and 360 min following the ZER or fentanest administration. The cut-off time was 25 s to avoid tissue injury.

Analgesímeter test

Four to five groups of mice, each one of them with about five animals, were studied. Both paws basal volume of all animals was measured, utilizing the LE 7306 Analgesimeter (Koster et al., 1959) apparatus. In the test of the induced ear edema, cróton and formalin capsaicina because of the zerumbona (1 mg ear, topical application) was evaluated for the inhibition of edema. According to the results, the zerumbone exhibited, in doses and routes tested analgesic effect in rats and mice *in vivo* and *in vitro*. The control group received 0.9% saline solution + Tween20 (10 mL/kg) and the positive received fentanest synthetic opiate (20 µg/kg) and indomethacin (25 mg/kg). One hour following gavage (v.o) or i.p administration, the edema was induced on the animals hind paws, by injecting a 1% carrageenan solution and, the same volume of 0.9% + Tween 20 saline solution in the contra-lateral paw.

Acute toxicity

The method described by Rocha and Silva (1968) and Salustiano et al. (1996) was employed. Mice were separated into ten, six-mice groups. They were fasted overnight and then were administered with the ZER in 10, 100, 1000, 1500, 2500 and 5000 mg/kg doses via intraperoneal and oral routes, while the control group only received the vehicle (0.9% saline + Tween 20). The mice were observed during 180 min for any abnormal behavior such as sedation, respiratory distress, motor impairment and hyper excitability and left to be observed 24 h, 48 h and 14 days later. Activities general tests were repeated in mice injected with identical doses and via the same routes.

Statistical analysis

All findings were acquired through statistical analyses of variance (ANOVA) with mean standard deviation and significance of $p<0.05$. The Student-Newman-Kelus test was adopted. The ID50 (dose that produced 50% inhibition in total time of paw licking) and 95% confidence intervals (CI) values were determined by using linear regression and graphs were drawn by using GraphPad Prism 4 software.

RESULTS

Structural determination of ZER from the essential oil of rhizomes *Z. zerumbet* (L.) Smith. The substance designated as zerumbone obtained from the recrystallization of essential oils from *Z. zerumbet* rhizomes was colorless, crystalline in appearance and the physico-chemical analysis of the substance showed a melting point of 64.5 to 64.57°C. Table 1 shows the 1 H NMR spectra exhibiting olefinic hydrogen signals at δ 5.25, 6.02, 5.99 and 5.87 with similar multiplicities.The purity of zerumbone was determined by high-performance liquid chromatography (HPLC), and was shown to exceed 99.95%. 13C NMR spectra showed 15 carbon signals, as 11 hydrogenated carbons (4 CH_3, 3 CH_2 and 4 CH), data provided by the DEPT-135 spectrum. Z. zerumbet rhizomes essential oils analyses findings confirmed the presence of a major sesquiterpene component called Zerumbone (Figure 1), which was characterized by UV-visible spectrophotometry, high efficiency liquid chromatography (HELC), by gas chromatography and identified through ^{1}H and ^{13}C NMR.

Table 1. Comparison between chemical shift signals obtained from 13C and 1HNMR spectroscopic data in the literature and zerumbone obtained from the essential oil of *Z. zerumbet* (L.) rhizomes.

13CNMR	Daí et al., (1997) $CDCl_3\delta$	Pinheiro (2005) Zerumbone and $CDCl_3$- 13CNMR		Hydrogen	Daí et al. (1997); $CDCl_3$ - ^{1}HNMR	Pinheiro (2005) Zerumbone 1HNMR
C-1	42.2	42.37	CH_2	H-1	1.9 (d,J=16 Hz); 2.2-2.5 (m)	1.9 (d; J=16 Hz); 2.17-2.36 (m)
C-2	125.0	124.97	CH	H-2	5.25 (dlJ= 16 Hz)	5.25 (dl; J=16 Hz)
C-3	136.1	136.21	C	-	-	-
C-4	39.4	39.39	CH_2	H-4	2.17-2.36 (m)	2.17-2.36 (m)
C-5	24.3	24.34	CH_2	H-5	2.2-2.35 (m)	2.17-2.36 (m)
C-6	148.5	148.74	CH	H-6	6.02 (dl;J=11 Hz)	6.02 (dl;J=11 Hz)
C-7	137.8	137.39	C	-	-	-
C-8	203.8	204.27	C=O	-	-	-
C-9	127.1	127.11	CH	H-9	5.93 (d;J=16,5 Hz)	5.99 (d;J=16,5 Hz)
C-10	160.4	160.68	CH	H-10	5.87 (d;J=16,5 Hz)	5.87 (d;J=16,5 Hz)
C-11	37.8	3780	C	-	-	-
C-12	15.2	15.14	CH_3	H-12	1.55 (s)	1.55 (s)
C-13	11.7	117.1	CH_3	H-13	1.80 (s)	1.80 (s)
C-14	24.1	24.13	CH_3	H-14	1.08 (s)	1.07 (s)*
C-15	29.4	29.37-CH_3	-	H-15	1.21 (s)	1.21 (s)*

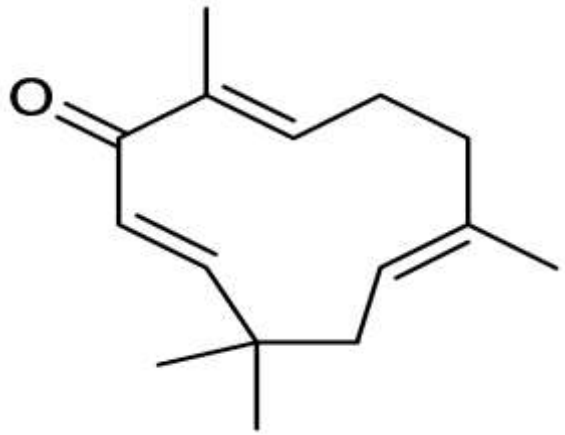

Figure 1. Chemical structure of zerumbone.

The 97% purity grade ZER was obtained through the national patent deposit process number PI0505343-9 (Pinheiro, 2005).

Pharmacological study

The effect of ZER on writhing response in mice is as shown in Figure 2. The ZER (150, 200, 220, 250, 500 mg/kg) given i.p. caused inhibition of dose-dependent acetic acid-induced writhing, with 98% of it being observed on the dose of 220 mg/kg (n=10, $p<0.05$) as compared to control. Such effects were also observed in mice pretreated with Fentanest (99%, $p<0.05$). Futhermore, the ZER (250, 500, 1,000, 1,500 mg/kg) given v.o. route 1 h before, ZER (1,000 mg/kg) caused a significant inhibition (98%) of the acetic acid-induced pain (Figure 2). The ED_{50} for ZER given via intraperoneal (i.p.) and oral (v.o.) routes, in this model, were 150, 6 (200-500) and (250-1,500 mg/kg), respectively. Through this test, it was established that ZER compound exerted a significant effect both via i.p. and v.o. by reducing the number of acetic acid-induced abdominal writhing contortions within 2 h. Essential oil from the rhizome of *Z. zerumbet* exhibited a significant antinociceptic effects on acetic acid-induced writing test in a dose-dependent manner, (Pinheiro, 2009).

From the Table 2, the administrations of the ZER (200-1500 mg/kg) v.o. and Fentanest (20 mg/kg) i.p. increased significantly the latency time to the nociceptive response in the hot plate test. In the antithermociceptive model (hyperalgesia), the treatment of animals with ZER (150-1500 mg/kg, p.i. or v.o.) altered the response to the stimulation up to 3 h following its administration (3.52 ± 0.21 min).

The application of fentanest (20 µ/kg, s.c) and ZER increased in 60% the time of reactivity (strength) of the stimulation application. Such outcomes demonstrate ZER to have central analgesic activity, similar to that presented by hypnoanalgesic drugs.

Formol-induced ZER analgesic activity test

The formalin test is one of the most widely used models

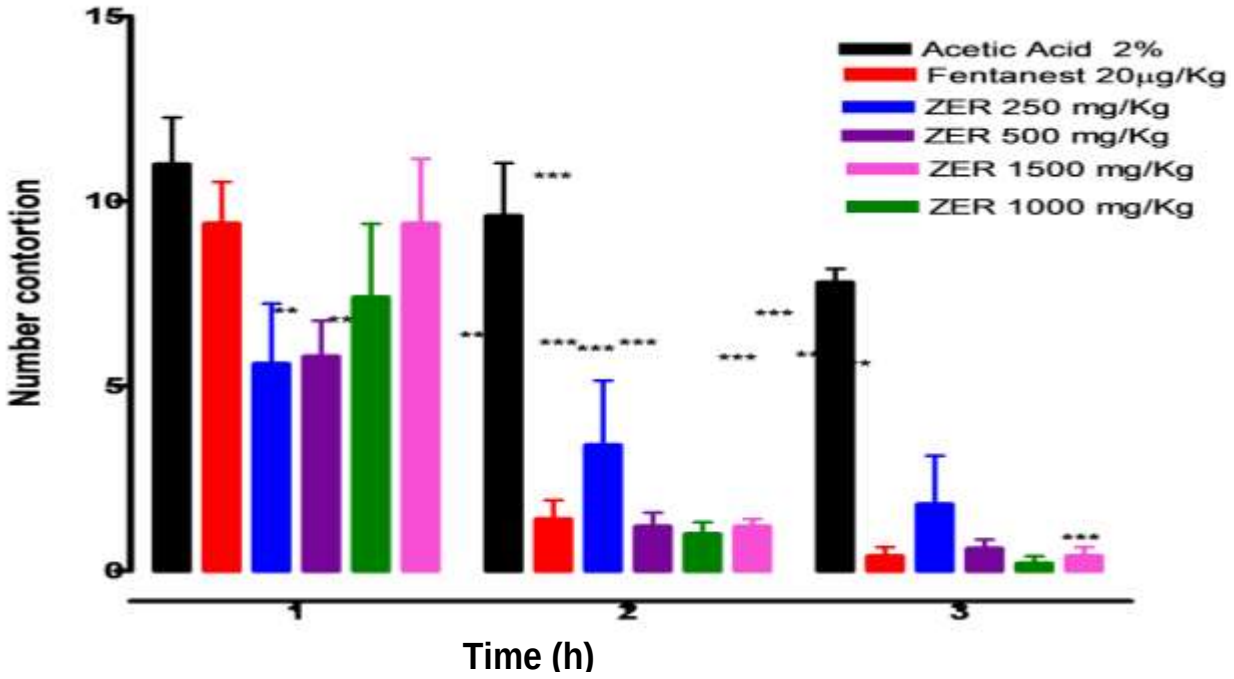

Figure 2. Percentage of oral inhibition (v.o), obtained in the acetic acid-induced writhing test and expressed for 3 h.

Table 2. Effect of the ZER substance on the hot plate test in mice.

Treatment	Reaction time (s)						
	Dose (mg/kg)	Via	30	60	90	120	180
Vehicle	-	i.p	4.10±0.36	3.82±0.35	4.68±0.55	5.70±0.36	7.45+0.16
Fentanest	20	i.p	3.62±0.58	3.62±0.61	8.78±0.41	9.38±0.57	10.70±0.26
ZER	250	v.o	6.39±1.10	7.59±1.98	10.18±2.06	10.86±1.08	8.01±2.33
ZER	500	v.o	8.09±1.29	11.20±2.26	12.67±2.33	13.45±1.55	14.51±0.49
ZER	1000	v.o	5.15±0.36	9.39±0.32	12.85±0.31	13.49±0.26	14.95±0.05
ZER	1500	v.o	7.18±0.39	14.39±1.34	15.05±0.39	15.49±0.29	15.95±0.85

Data are average ± EPM; $p<0.05$ in relation to control; $p<0.01$ in relation to control; n = 10.

to explain pain and analgesia mechanisms, with better results than those using mechanical or thermal stimuli. The model consists of two distinct phases. The first phase represents the irritant effect of formalin on the sensory C-fibers (Figure 3).

The formalin nociception model consists of the intraplantar injection of formaldehyde solution in the hind paw of the animal, which triggers intense nociception by direct stimulation of the nociceptors. Nociception caused by intraplantar injection of formalin and characterized by vigorous licking, biting and beating of the injected paw as an irritant. Most studies using this model use rodents, predominantly mice and mice. This test is characterized by two distinct stages of nociception, which seem to involve different mediators (Hunskaar et al., 1985; Hunskaar and Hole, 1987; Correa and Calixto, 1993). The first phase of nociception begins immediately after formalin injection, extending for the first 5 min, which is believed to be due to direct chemical stimulation of the nociceptors (Hunskaar et al., 1985), predominantly of type C afferent fibers and partly of type (Heapy et al., 1987) and is associated with the release of excitatory amino acids, nitric oxide and P-substances between others.

Figure 4 shows the second stage of this model occurs between 15 and 30 min after formalin injection and is mainly related to the release of several pro-inflammatory mediators such as bradykinins, histamine, prostaglandins and serotonins, among others (Hunskaar and Hole, 1987; Correa and Calixto, 1993).

The present results from the present thesis showed that, when evaluated in the formalin test, the

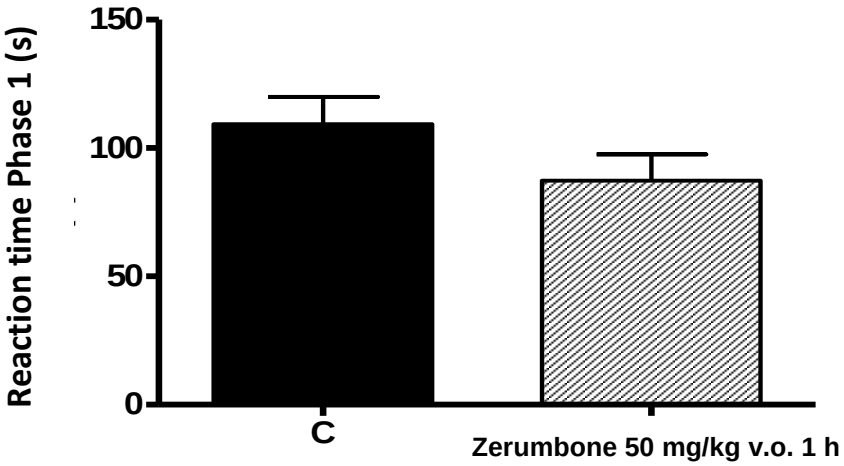

Figure 3. Effect of the product ZER (zerumbone) 50 mg/kg v.o. 1 h in the model of 2.5% formalin-induced pain (first stage) in mice.

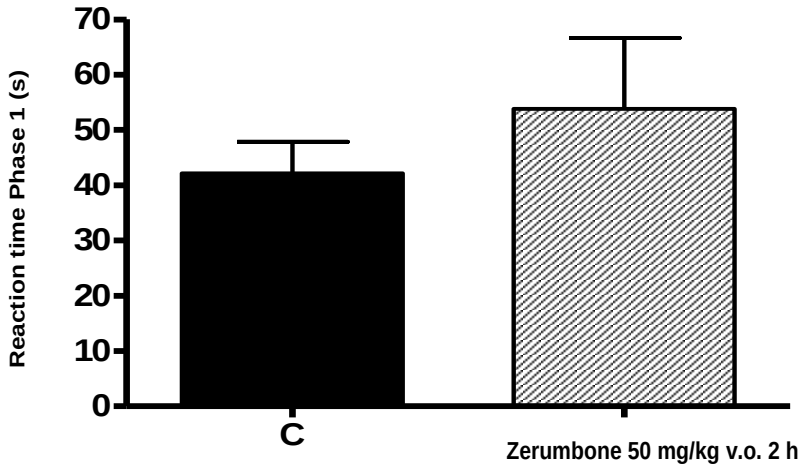

Figure 4. Effect of the product ZER (zerumbone) 50 mg/kg vs. 2 h in the pain model induced by 2.5% formalin (second stage) in mice.

sequiterpenico ZER administered orally caused maximum antinociceptive activity in 1 h, being significant within 6 h after its administration, in relation to the second phase of the formalin induced nociception. In addition, the present data also demonstrated that the ZER compound, administered v.o, produced a significant antinociceptive effect in a dose-dependent manner in relation to both phases of formalin-induced nociception, but was more effective in relation to the second phase of this model. Thus, the results reinforce the hypothesis of the important antinociceptive and/or anti-inflammatory activity.

Analgesic activity produced by capsaicin

The treatment of animals with the ZER sesquiterpene (1-3%) caused significant reduction and dependent neurogenic pain induced by ear edema intraplaque injection of capsaicin. The results indicate that ZER sequiterpene at the dose of 50 mg/kg when compared with the control inhibited 70 + 5% of pain caused by capsaicin.

The results obtained in the model revealed that ZER sequiterpene, administered v.o, was able to inhibit neurogenic pain caused by capsaicin, thus opening up new perspectives for the therapeutic use of the ZER compound in these types of pains. Another important aspect in the present study was the fact that ZER showed the same efficacy and potency both administered orally and i.p. suggesting that this sesquiterpene has good availability, which makes this compound important for a future drug with analgesic activity (Figure 5).

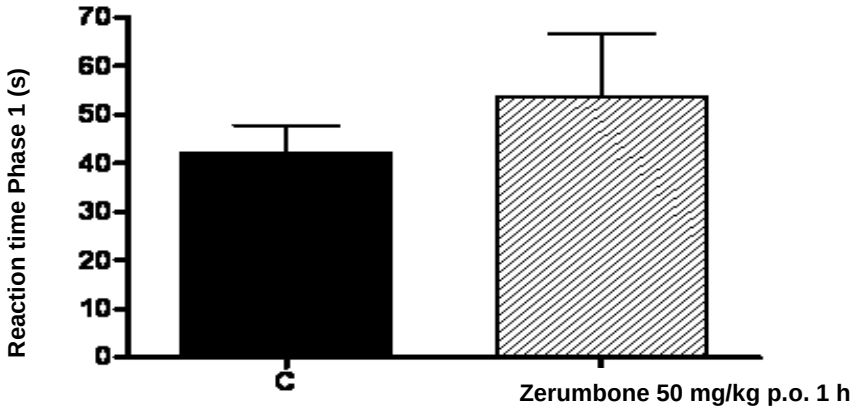

Figure 5. Effect of the zerumbone (ZER) Product (50 mg/kg p.o.) 1 h in the induced pain model by capsaicin 1.6 µg/paw in mice.

DISCUSSION

The present study shows clearly the sesquiterpene ZER obtained from *Z. zerumbet* rhizomes essential oil to possess potent antinociceptive activity when administered via intraperitoneal or oral routes in the different neurogenic-induced nociception models in mice and rats. Moreover, the compound presented expressive antinociceptive activity when analyzed in the hot plate thermal sensitivity assessment nociception. Among the pain models used in this work, that of acetic acid intraperoneally-induced abdominal writhing is relatively simple with little specificity, but easy to be observed, quick and with good sensibility to several non-steroidal antiinflamatory and analgesic drugs, as well as to drugs similar to other centrally-acting analgesics.

Through this test it was possible to demonstrate, for the first time, that ZER administered via oral route, inhibits acetic acid induced writhing up to 98% within 2 h. In an *in vivo* assay, an acetic acid-induced writhing response in mice was significantly reduced by treatment with zerumbone. Furthermore, zerumbone reduced paw edema and the pain response in a mono-iodoacetate (MIA)-induced rat osteoarthritis model (Chien et al., 2016). Sulamain et al. (2010) tested ZER in doses of 10, 50 and 100 mg/kg with realized inhibitions of 19.3, 40.4 and 64.8%, respectively as compared to the control (acetylsalicylic acid). It was concluded that the direct acetylsalicylic acid induction can liberate endogenous mediators, with prostaglandins E2 (PgE2) and PGF2 in peritoneal fluids. While ZER inhibits abdominal writhing through the lipoxygenase and/or cycloxygenas mechanism by reducing the afferent nociceptors primary transduction. Expriments carried out with pharmacological agonists and antagonists suggest that the analgesic effect caused by substance ZER is probably related with an interaction with the opiate or glutaminergic systems without involving the L-arginine/nitric oxide pathway and sedative or muscle relaxing effects on the central and/or peripheral nervous system.

Another major aspect being analyzed in the present study is the possibiliy of the sesquisterpene ZER to produce analgesic effect in the supra-spinal mechanism in a nocicepuion model, which utilizes harmful thermal stimulation in the experiments using the hot plate. Doses that showed to be effective in the hot plate test, at 50°C, were similar to those that were necessary and efficient for the analgesic effect in chemical stimulation model. Furthermore, the present study demontrates the positive control, fentanest (synthetic opiate), to be effective in also lengthening the time of latency of the animals in the thermal nociception stimulation, regardless the temperature being used. That might be a reflex either from the great difference of the stimulations, or from the integration of the responses to different temperatures (Hwang and Wilcox, 1987).

Sulamain et al. (2010) investigated the effects of ZER through the test of thermal stimilations (hot plate) following the protocol for intraperitoneal administration (10, 50 and 100 mg/kg) with a special significance in the dose of ZER having a prolonged latency in the heat stimulation. The ZER effective time in mice using the hot plate test also confirmed its anti-nociceptive activity through its action on the central mediators. Perimal et al. (2011) demonstrated that zerumbone possesses significant peripheral and central antinociceptive effects.

Given that neurogenic nociception is very complex and that there are no satisfactory therapeutic alternatives for its treatment, as of yet, these findings may open new perspectives for the development of molecules presenting therapeutic potential for treating pain (Carter, 1991; Julius and Basbaum, 2001; MacFarlane et al., 1997).

Conclusion

The findings of the present study suggest ZER in the models of acetic acid-induced nociception, thermal and

mechanical tests: hot plate and hyperalgesia to present therapeutic potential, contributing to the discovery of new drugs possessing analgesic effect, so much so as to be referred to for the control of the neurogenic nociception. Another important feature in the present study was the fact of ZER presenting the same efficacy and power, when administered in dependent dose both via oral and intraperitonial route, suggesting this substance to present good availability.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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Study of oxygen transfer processes improvement for domestic wastewaters treatment

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In aerated processes, the oxygen transfer was limited by the presence of the suspended matter as colloid and the dissolved matter, which might decrease the biological degradation effectiveness. In this publication, three series of tests were conducted to study possibilities to reduce these matters: bacterial adaptation which was conducted in a biological aerated filter, adsorption/biosorption conducted on a biological aerated filter with a biofilm of adapted bacteria and percolation in a bioreactor with a packed plastic media. All the tests carried out gave convincing results concerning turbidity and chemical oxygen demand, as parameters limiting the oxygen transfer for a better biodegradation. The advanced adaptation improved their elimination. So, all these treatment techniques could be used as pretreatment processes; in addition, they required very little energy, particularly adsorption/biosorption and percolation.

Key words: Pretreatment, purification, biological aerated filter, adsorption, biosorption, percolation, packed plastic media.

INTRODUCTION

Domestic wastewaters posed a serious problem in arid and semi-arid regions of the Middle East and North Africa, including Morocco, on the environment and health, resulting in several negative impacts. Water quality degradation is quickly joining water scarcity in most countries of these regions (Chaoua et al., 2017). To deal with this problem, the Biological Aerated Filters (BAF) representing the most economical upgrading technology, have been put in place.

BAF is a flexible reactor, which provides a small footprint process option at various stages of the wastewater treatment (Farabegoli et al., 2009). It

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contains a granular media with high surface area allowing bacteria to develop as a biofilm. This bioreactor was widely used in aerobic wastewater treatment and provided excellent purification performances (Chaudhary et al., 2003; Datta and Allen, 2005; Papadia et al., 2011; Pramanik et al., 2012).

Oxygen was the key element in the aerobic biological purification process. However, when it is limited, the kinetics of biological degradation decreased. This might be due to the presence of the colloidal and the dissolved matters present in the wastewater (Seo, 2011). Their prior elimination would improve the biological degradation process, by enhancing the oxygen transfer.

In the presence of the biofilm, colloidal matter can be eliminated by adsorption. Dissolved matter can be removed by a complex mechanism involving convection, diffusion, adsorption, hydrolysis and finally, the degradation reaction itself (Picard, 2011). Some pollutants like heavy metals, dyes and refractory organic matter could be removed by biosorption through absorption, adsorption, and ion exchange (Gadd, 2008). The role of the biofilm in purification was more accentuated when the bacteria were adapted, because their increasing population allowed them to further colonize the surface area (kumar et al., 2013; Roux and Ghigo, 2006; Vargas, 2013).

During wastewater percolation in a trickling filter, some dissolved gases could be removed by the physicochemical way, especially through spreading the wastewater over the packed plastic media in a thin film. This spreading also promoted a better contact between the biofilm formed on the packed plastic media and the dissolved matter and consequently increased the biodegradation of the organic matter and the retention of the colloidal matter (Metahri, 2012; Racault and Seguret, 2004).

The objectives of this study were to show the treatment capacity of BAF, purification performance on the elimination of organic pollution and suspended solids of domestic wastewaters that came from a suburban site located about 15 km from Casablanca.

MATERIALS AND METHODS

Presentation of the study area

The wastewater used in this study came from a suburban area not connected to a municipal network, 15 km located from the Casablanca city. This wastewater has been harvested from an open-air ditch, put in a can, and immediately transported to the laboratory for analysis and tests.

Description of the experimental pilot

Tests of adaptation were performed in a PVC pilot as a biological aerated filter, operating in batch mode (Figure 1a). It consisted of a cylindrical column of height of 70 cm and a diameter of 10 cm. It was fulfilled by a packing media P1 (Figure 1b), with characteristics shown in Table 1. It was aerated by an aerator made of rigid expanded polyurethane like a diffuser (length of 9 cm and width of 1 cm), placed at the bottom of the BAF, and giving an air flow rate of 0.5 L/s. The sludge detached from biofilm during the biological process was evacuated from the BAF by a purge valve located under the aerator. The sampling was made from a valve located at the top of the bioreactor. A grid was placed above to fix the packing media to prevent its flotation by the air bubbles coming from the diffuser.

Tests of adsorption/biosorption were made in the same reactor that was used for adaptation tests, but without aeration. For percolation, tests were performed in the same bioreactor and were operated in continuous mode. It was fulfilled of packing media P1, P2 and P3 (Figure 1b), with characteristics shown in Table 1. The characteristics of wastewaters used in different series of tests are shown in Tables 2, 3, and 4.

Monitoring of parameters and used material

During different tests, the monitoring of the treatment performances was conducted with the following physicochemical parameters: chemical oxygen demand (COD), dissolved oxygen (DO), pH, turbidity and conductivity. The COD and turbidity were determined by the Palintest 7000 type photometer. The dissolved oxygen was measured by an oxygen probe connected to the oximeter (Hach 40d-HQ multi oximeter). The pH was measured by the same device. The conductivity was determined by using the conductimeter (Orion model 125).

The bioreactor performances could be expressed by the abatement rate and abatement, respectively by the following equations:

$$\boldsymbol{Y}\ (\%) = (\boldsymbol{L_0} - \boldsymbol{L})/\boldsymbol{L_0} \quad (1)$$

$$\text{Abt} = \boldsymbol{L_0} - \boldsymbol{L} \quad (2)$$

Where, $\boldsymbol{L_0}$, is the initial value (Turbidity, COD), $\boldsymbol{L}$ is the initial value (turbidity, COD), Y is the abatement rate, and Abt is the abatement (Turbidity, COD).

Operating protocol

In the laboratory, three series of tests were done:

(1) Adaptation of the bacteria through seven tests, conducted in a biological aerated filter, fulfilled by a packing media operating in batch mode. After each test, when the COD reached maximum elimination, the residual reject was evacuated from the BAF and replaced by a new domestic wastewater solution more concentrated. This operation was repeated several times until it reached good bacteria acclimatization. The characteristics of the rejects used during different adaptation tests are shown in Table 2.
(2) Adsorption/Biosorption tests were conducted in a non-aerated BAF with packing media, colonized by adapted bacteria and operating in batch mode. Four tests were performed. For each one, when the adsorption/biosorption reached its maximum, the wastewater was removed from the bioreactor. It was then replaced by a new solution. Table 3 shows the characteristics of wastewaters used in the adsorption/biosorption tests.
(3) Percolation of wastewaters in a trickling filter. The tests were carried out on three clean non-colonized packing media, with three rates for each one: 0.106, 0.212 and 0.318 m/h. The characteristics of wastewaters are shown in Table 4.

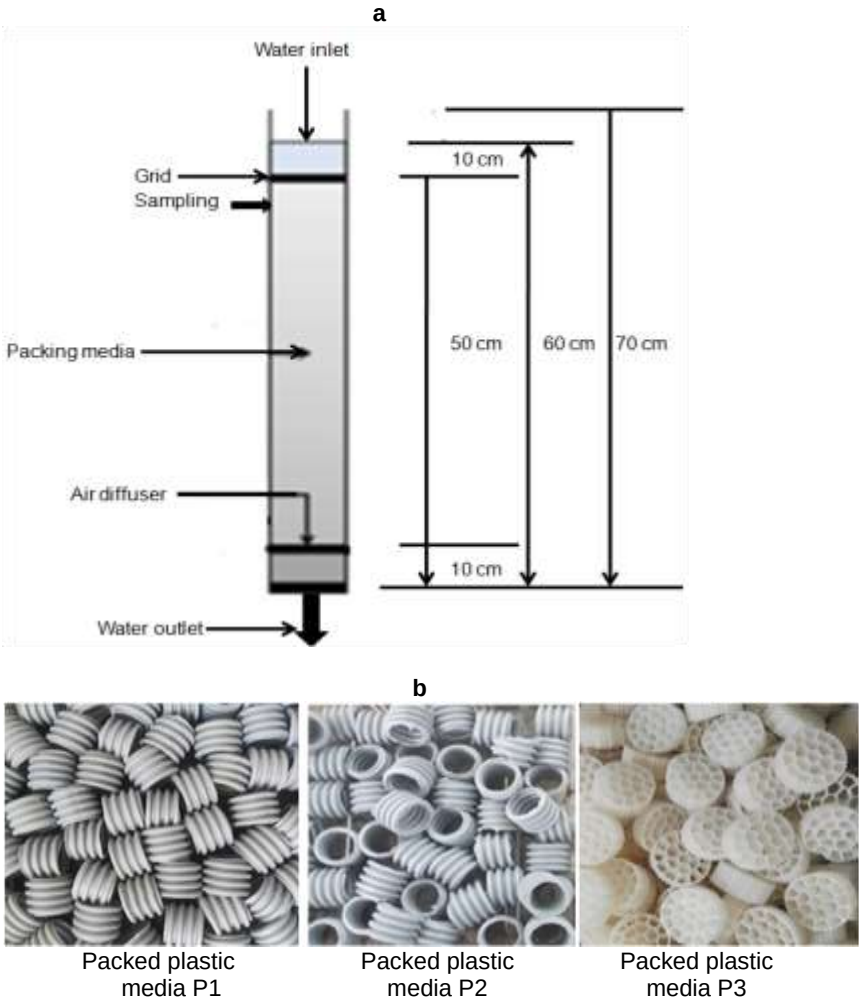

Figure 1. (a) Experimental pilot. **(b)** Packing used in the reactor for the three experiments.

RESULTS AND DISCUSSION

Adaptation tests

The COD decreased during time and during the different adaptations essays (Figure 2a). The abatement rate of COD (Figure 2b), improved further when the bacteria responsible of the biodegradation reached a high degree of adaptation (Figure 2c).

The improvement of COD abatement was corroborated by some authors (Kherbeche et al., 2017; Lele and Watve, 2014; Pramanik et al., 2012; Amrouche et al., 2011). They have shown that the abatement rate improved with the increase of the initial concentration of the COD, in adaptation tests realized on synthetic solutions of glycerol as an organic substrate. Similarly, Kherbeche (2016) showed that, in comparative tests between two BAFs, one was fed by domestic wastewater and the other by domestic wastewater with increase in COD; the kinetics of biodegradation improved more when COD is increased.

Concerning the turbidity, the wastewater presented variable initial values (Table 2). During the adaptation tests, it decreased (Figure 3a). Figure 3b shows the elimination rate; it is depended on the adaptation degree during the first hours, but then it reached about 97% after 96 h for the whole adaptation tests.

The elimination of the turbidity was due to the suspended matter retention in the bioreactor, which probably occurred because of (1) the physical interception phenomena by the packing media and/or (2) the bioflocculation by extracellular polymeric substances (EPS) of the biofilm, increasing further when bacteria adaptation is improved (Lekhlif et al., 2015; Hongyuan and Wenchao, 2013; Boltz et al., 2006; Sheng et al., 2010).

The conductivity presented in Figure 4a varied according to the adaptation degree and gave values from

Table 1. Characteristics of the packing media.

Parameter	Packing media P1	Packing media P2	Packing media P3
Color	Grey	Grey	White
Diameter (mm)	19.5	15	25
Length (mm)	15	15	12
Specific surface (m^2/m^3)	~419	~869	~427
Porosity (%)	85	87	92,5

Table 2. Characteristics of wastewater during adaptation tests.

Parameter	Adapt 1	Adapt 2	Adapt 3	Adapt 4	Adapt 5	Adapt 6	Adapt 7
Dilution ratio	5	5	3	2	1	1	1
L_0 (mgO_2/L)	560	590	1000	1750	2700	3000	3200
DO (mg/L)	7.07	0.42	0.47	0.29	0.29	0.49	0.33
Turbidity (NTU)	170	160	280	150	550	750	625
Cond (µS/cm)	2.06	2.39	4.35	3.11	5.91	6.17	6.12
pH	6.56	6.91	6.73	5.91	6.72	6.77	7.23
T°C	18.1	19.1	17.6	18.9	17.9	17.4	18.2

Table 3. Characteristics of wastewater during the adsorption/biosorption tests.

Parameter	Peripheral zone wastewater of Casablanca			
	Essay 1	Essay 2	Essay 3	Essay 4
L_0 (mgO_2/L)	3000	2200	1800	1550
DO (mg/L)	0.3	0.31	0.31	0.27
Turbidity (NTU)	525	580	520	440
Cond (µS/cm)	6.01	5.99	6.02	6.09
pH	7.28	7.42	7.28	7.4
T°C	19.1	19.4	19.2	20.2

Table 4. Results of the different trickling filter tests on the packed plastic media at different Peripheral rate (m/h)

Parameter	P1			P2			P3		
Peripheral speed (mL/min)	0.106	0.212	0.318	0.106	0.212	0.318	0.106	0.212	0.318
L_0	2400	2700	2700	2400	2600	2600	2200	2200	2700
L	750	950	1400	1400	1500	1400	1250	1250	1050
COD yield	68.7	64.81	48.14	41.67	42.3	46.15	43.18	43.18	61.11
Initial turbidity	900	900	520	400	410	500	380	410	380
Final turbidity	420	440	380	210	170	160	120	170	140
Turbidity (Y %)	53.3	55.5	26.9	47.5	58.5	68	68.4	58.5	63.1
DOi	0.23	0.21	0.27	0.29	0.28	0.22	0.31	0.31	0.31
DOf	0.24	0.24	0.34	0.97	0,19	0.16	0.41	1.02	0.31
pHi	7.29	7.29	7.45	7.47	7.87	8.17	8.11	8	7.95
pHf	8.34	8.37	8.26	8.7	8.49	8.53	8.73	8.34	8.41

7.07 to 9.33 µS/cm for the first adaptation, and values varying between 2.26 and 6.17 µS/cm for the other adaptations. The conductivity increase for the first adaptation could be explained by the hydrolysis of long-

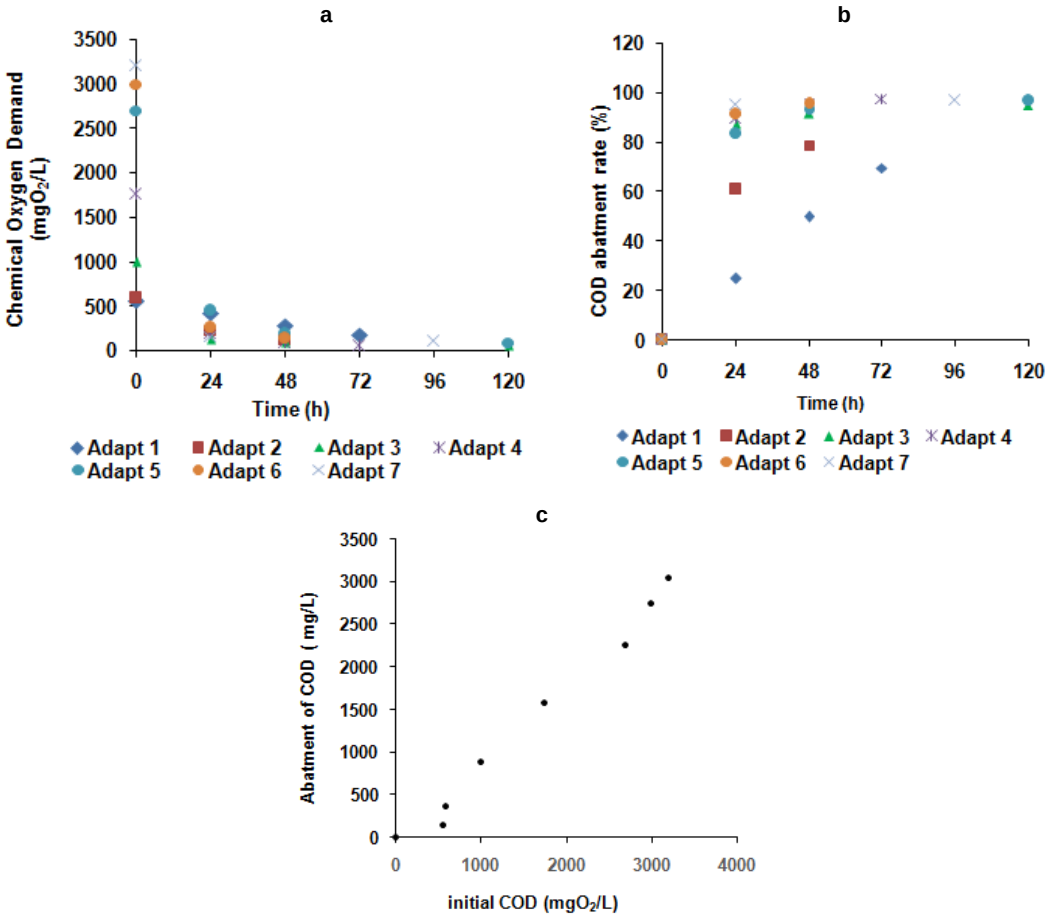

Figure 2. (a) COD evolution during the different adaptations. **(b)** Evolution of the rate of reduction COD during the various adaptations. **(c)** Evolution of the rate of elimination of the COD as a function of the initial concentration after 24 h.

chain organic matter into little molecules which involved conductivity increase, and in the same time the absence of sufficient biomass that allowed the elimination of solution ions, including metal cation, by biosorption to colonize it. The conductivity decrease was probably due to some phenomena: biosorption of the organic matter by biofilm extracellular polymeric substances, reduction of the mineral fraction by biological reaction as trace elements, complexation of metals by the hydrolyzed organic matter and their biosorption on the biomass with formation of metal bridges and/or metals precipitation resulting from the pH increase (Figure 4c) (Prieto et al., 2002).

As shown in Table 2, it was noted that except for the 5 times diluted wastewater, with initial DO of 7.07 mg/L (adaptation 1), the DO for the other samples was low, it was between 0.29 and 0.47 mg/L. These low values were both due to the dissolved matter whose presence increased the viscosity of water (Chern et al., 2001; Jimenez et al., 2013) and to the suspended matter which affected negatively the gas-liquid oxygen transfer (Kuan, 2009). With time and the various adaptations, this (Kherbeche, 2016). Indeed, during the first adaptation, characterized by slow biodegradation kinetics (Figure 2a and 2b), the packing plastic media were clean and the bacteria took some time to adapt their enzymes, and then concentration increased (Figure 4b). For a long time, it reached about 9 mg/L for all the tests, because of the reduction of suspended and dissolved matters.

The pH of the different solutions increased for all adaptations (Figure 4c). This behavior has been noted by other authors (Kherbeche et al., 2017; Pakanati et al., 2018). This could either be due to the elimination of CO_2 by aeration or to the denitrification that could be installed in the biofilm, especially when its thickness increased. ET-taleb et al. (2014) and Wicke et al., (2007) mentioned that the organic matter decomposition in anaerobic conditions led to the increase of pH. Otherwise, Oehmen et al., (2005) have shown that this increase could be explained by the consumption of nitrogen compounds during the reactor aeration. They have also shown an identical pH behavior after 2 h of aeration in a sequencing batch reactor. Jeong et al. (2008) noted the same observations.

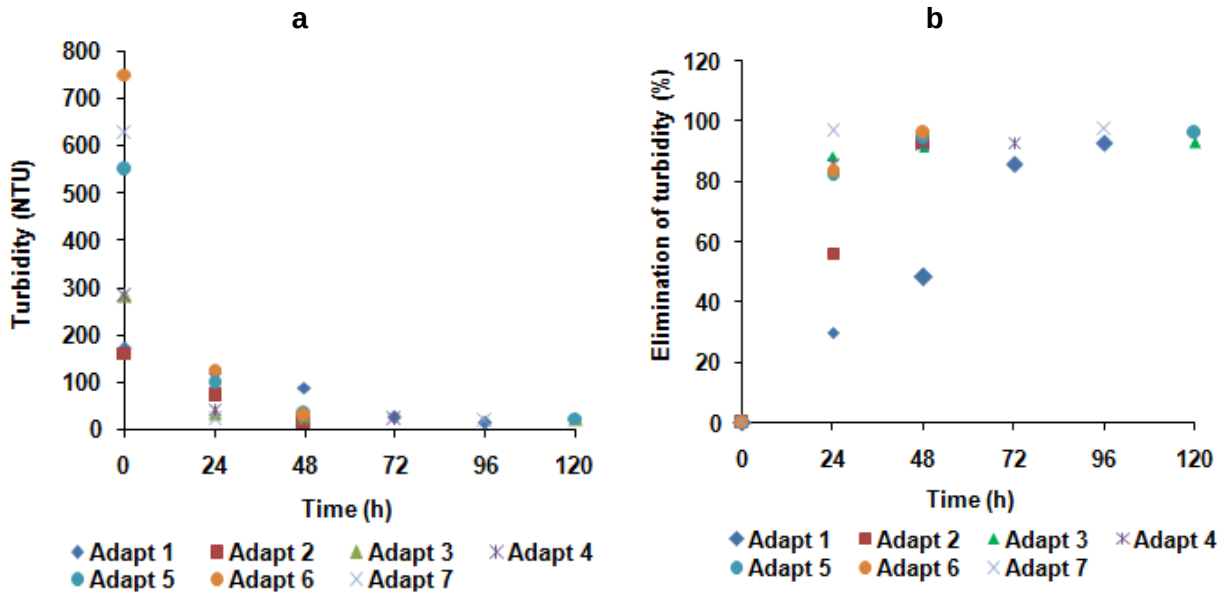

Figure 3. (a) Turbidity evolution. **(b)** Evolution of elimination rate of the turbidity.

Adsorption/Biosorption tests

Figure 5a and 5b shows respectively both the evolution of the COD and its abatement rate for the four tests. The COD reduced probably because of the dissolved matter retention on biofilm by biosorption. It was better when the COD was high (Figure 5c). This could be probably explained by a better matter transfer by diffusion in the boundary layer, which was created (in the absence of agitation) between the liquid phase and the biofilm (Picard, 2011; Lé, 2008).

Figure 6a shows that turbidity was eliminated for all tests. Nevertheless, when its values increase, its abatement rate decreased (Figure 6b). This could probably be explained by the limited biofilm capacity to absorb efficiently the colloidal matter. The eliminations of colloidal and dissolved matters seemed not to be the same way. They occurred according to different mechanisms. Colloidal matter was eliminated by adsorption on the limited surface area of the biofilm, whereas dissolved matter was eliminated by biosorption on a high surface area including that of the internal porosity of the biofilm.

The conductivity varied very slightly for all the tests (Figure 7a), in a range between 10 and 80 µS/cm. This small variation was probably the consequence of concomitant reactions which have contradictory effects: (1) the elimination of electrical entities was present in solution by several phenomena: biosorption of organic ionized molecules of metals (Lé, 2008; Jeong et al., 2008), metal complexes, and formation of metal bridges at the biofilm (Et-Taleb et al., 2014), and (2) endogenous respiration, which could release ions in solution following the destruction of microorganisms or the resolubilization of some metal precipitates when the pH increased (Figure 7c).

Figure 7b shows that the concentration of dissolved oxygen varied between 0.26 and 0.63 mg/L. Its values remained more or less constant, particularly for the second and the third test. It improved in the fourth test after 2 h, probably because of the dissolved matter retention in the biofilm and the bioflocculation of the suspended matter.

For pH, there was at first an increase for all the tests and then stabilization around an average value of 7.7 (Figure 7c). This was the same observation noted in the previous adaptation tests. The increase in pH might be due to the denitrification process (Oehmen et al., 2005; Horan, 2003).

Percolation tests

The percolation tests, giving the results presented in Table 5a, b and c, showed that COD and turbidity were substantially reduced. The removed COD corresponded probably to the gaseous compounds, such as H_2S and other volatile organic compounds dissolved in the wastewater. Turbidity decreased due to retention of the suspended matter on packing media P1, P2 and P3, probably by interception and decantation on its high surface area.

The removal rates depend on the percolation rate and the packing media type. It varied between 26.9 and 68.4% for turbidity and between 48.14 and 65.90% for COD.

Conductivity decreased slightly in all tests (Figure 8a). This could probably be justified by the volatile matter

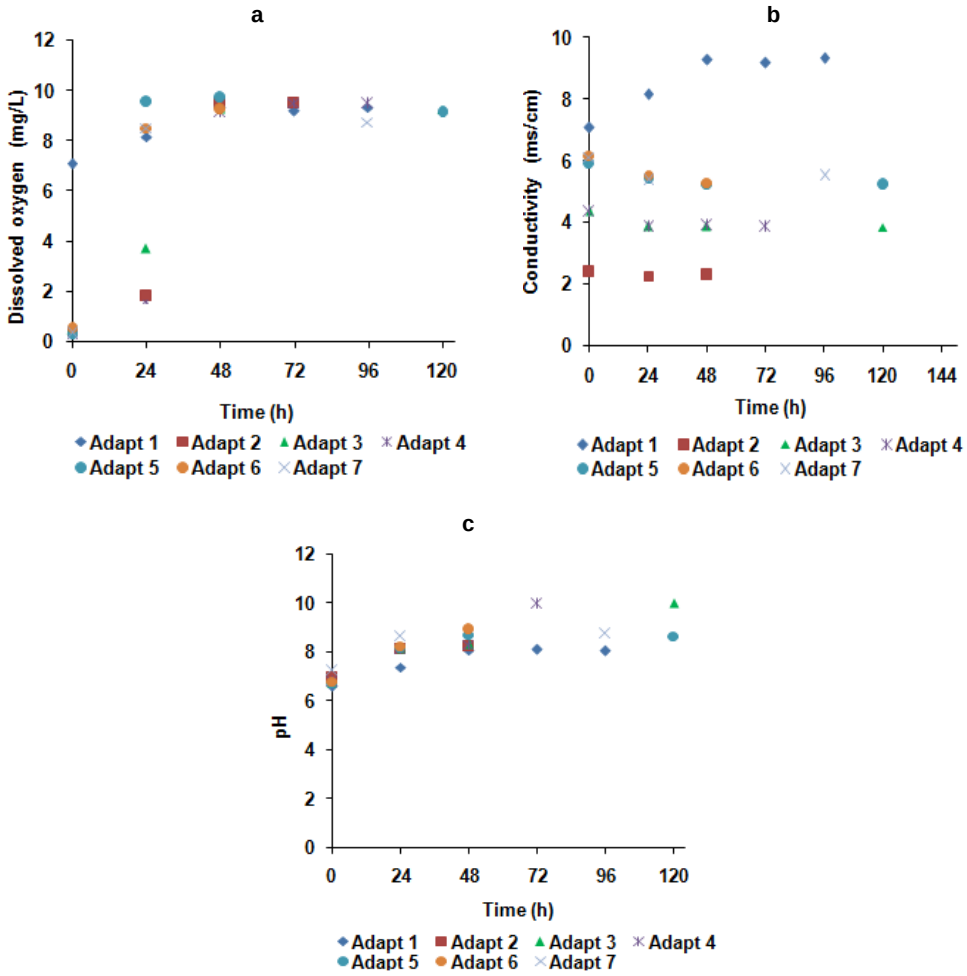

Figure 4. (a) Evolution of the dissolved oxygen. **(b)** Evolution of the conductivity dissolved oxygen. **(c)** Evolution of pH, according to the degree of adaptation.

elimination, in particular H_2S which resulted in a shift of chemical equilibrium to hydrogen sulphide formation (Reactions 1 and 2).

$$H_2S \Leftrightarrow H^+ + HS^- \quad (1)$$

$$HS^- \Leftrightarrow H^+ + S^= \quad (2)$$

This could be confirmed by the pH increase during the various tests (Figure 8b), resulting from the elimination of H_2S.

Conclusion

All the tests carried out in the three series gave convincing results concerning the limiting parameters of the oxygen transfer: the suspended and dissolved matters. The adaptation tests have shown that the elimination of these two parameters improved further with the degree of adaptation. The biosorption and the percolation tests gave interesting elimination rates. So, they could be used as a pretreatment process. In addition, they required very little energy.

These tests, performed separately, showed more or less similar yields, but they were differentiated in terms of treatment time. The percolation performed on a clean packed plastic media seemed to have better treatment dispositions; under real operation, the clean packed plastic media would be colonized by adapted bacteria, so that they could further be involved high treatment performance. In addition to percolation, the phenomenon of biosorption would also occur. This could substantially improve the elimination rate of suspended and dissolved matter and make better the oxygen transfer.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

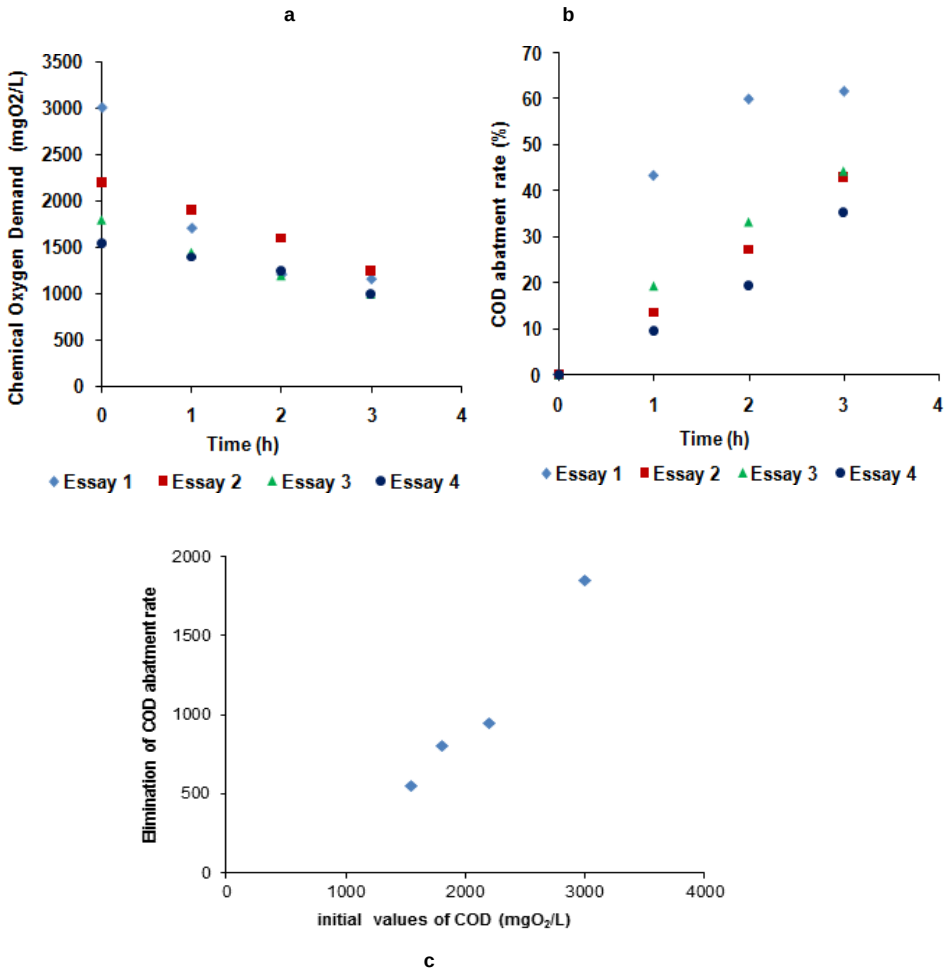

Figure 5. (a) Evolution of CO. **(b)** Evolution of the COD abatement rate. **(c)** Evolution of the COD abatement during, biosorption tests after 3 h.

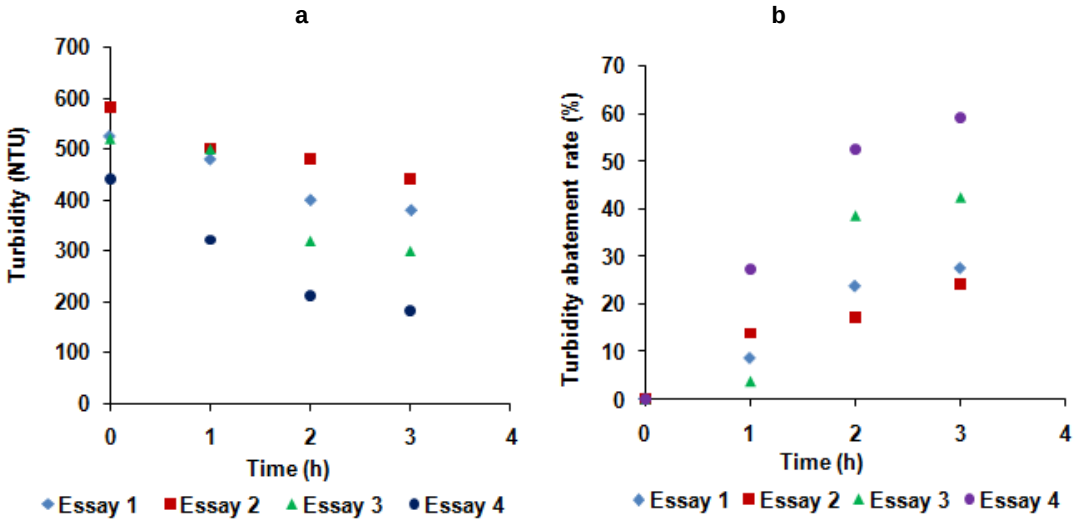

Figure 6. (a) Evolution of the turbidity. **(b)** Evolution of the abatement rate of the turbidity. **(c)** Evolution of the turbidity abatement, during biosorption tests after 3 h.

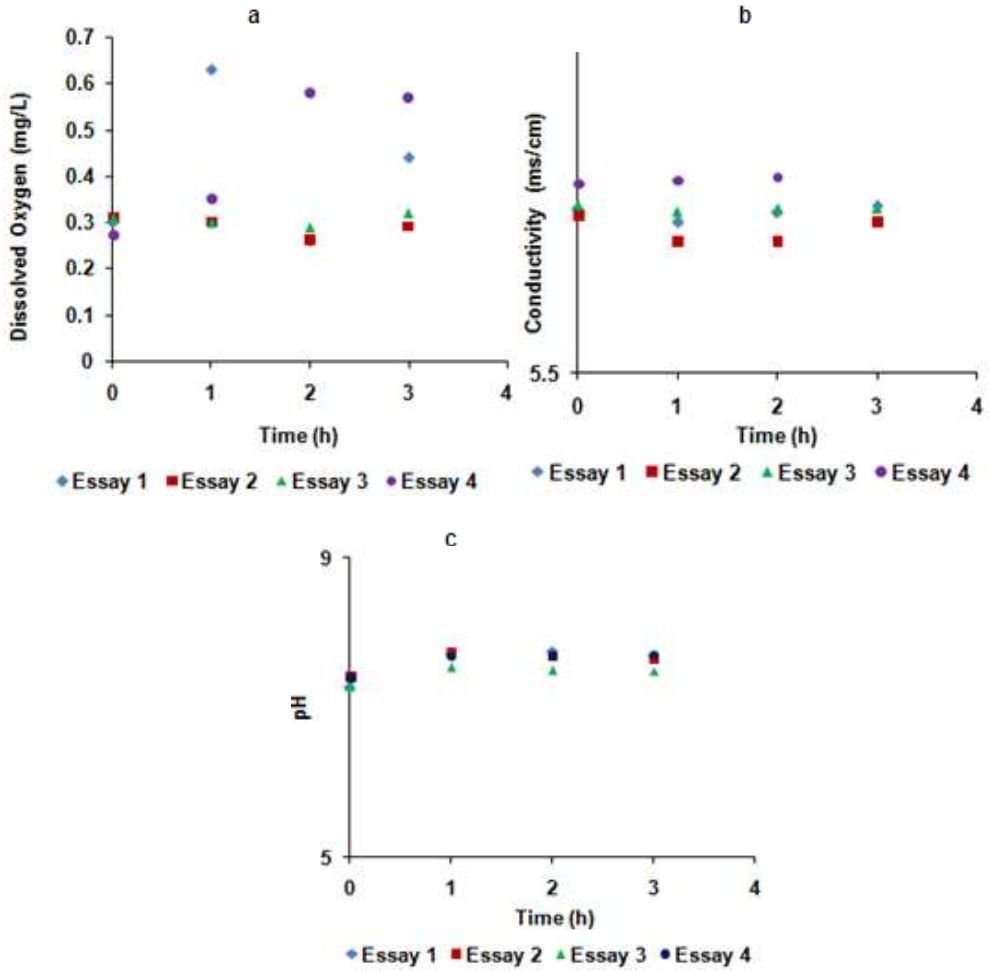

Figure 7. (a) Evolution of conductivity. **(b)** Evolution of dissolved oxygen. **(c)** pH Evolution.

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