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Enhanced hexadecane degradation and low biomass production by *Aspergillus niger* exposed to an electric current in a model system

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ABSTRACT

The effects of an electric current on growth and hexadecane (HXD) degradation by *Aspergillus niger* growth were determined. A 450-mL electrochemical cell with titanium ruthenium-oxide coated electrodes and packed with 15 g of perlite (inert biomass support) was inoculated with *A. niger* (2.0×10^7 spores (g of dry inert support)$^{-1}$) and incubated for 12 days (30 °C; constant ventilation). 4.5 days after starting culture a current of 0.42 mA cm^{-2} was applied for 24 h. The current reduced (52 ± 11%) growth of the culture as compared to that of a culture not exposed to current. However, HXD degradation was 96 ± 1.4% after 8 days whereas it was 81 ± 1.2% after 12 days in control cultures. Carbon balances of cultures not exposed to current suggested an assimilative metabolism, but a non-assimilative metabolism when the current was applied. This change can be related to an increase in total ATP content. The study contributes to the knowledge on the effects of current on the mycelial growth phase of *A. niger*, and suggests the possibility of manipulating the metabolism of this organism with electric current.

1. Introduction

The behavior of microorganisms exposed to an electric field has been studied for decades, especially with the aim of analyzing the loss of viability, mobility, and the resulting changes in microbial metabolism, including permeability (Teissié et al., 2002; Virkutyte et al., 2002). The outcomes of application of electric currents depended on the current intensity and ranged from lethal to minor metabolic modifications (Thrash and Coates, 2008). When high voltages were applied, sterilization can be achieved (Teissié et al., 2002). This inactivation of microorganisms is either due to thermal effect as a result of the ohmic drop associated with the electric potential (Palaniappan et al., 1992) or to the production of toxic substances generated by reactions at the electrodes, such as soluble metallic ions and formation of free chlorine (Guillou and El Murr, 2002). Tests on yeast cells (*Candida utilis, Hanseniasapora guilliermondii and Saccharomyces cerevisiae*) using 40 kW cm^{-1} and bacterial cultures showed a significant decline in microbial viability because of changes in cell morphology (Palaniappan et al., 1992). Under a high direct electric current, electrophoretic mobility of bacteria is related to zeta potential, *i.e.* the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle charge surface on the cell (Jackman et al., 1999). An irreversible permeability phenomenon at the cell membrane has been observed in yeasts upon applying a high-intensity electric current (2.75 kV cm^{-1}; Ganeva et al., 2002).

Low-intensity electric current application (lower than 5 mA cm^{-2}), has been used to control the odor and hygienic state of wastewater slurries (Zanardini et al., 2002) and to reduce or eliminate undesirable microbes present in food preparation processes and conservation (Bawcom et al., 1995). Such currents have been shown to stimulate both the production of secondary metabolites (Shin et al., 2002), and degradation of organic molecules (Hyo-Sang and Lee, 2001). For example, when low-intensity electric current (1.98 mA cm^{-2}; 21 h) was applied on two different soil microbial consortia, including fungi, an increase in the carbon source (phenol; 1.2 g L^{-1}) consumption was observed (Xin-gang et al., 2006). In fungi and bacteria, the CO_2 production rate increased by as much as 290% (0.314 mA cm^{-2}; 27 d) when six different carbon sources were assayed. Moreover, fungi and bacteria counts in cultures with and without electric current showed no significant differences (Lear et al., 2004). Although most explanations are correlated to membrane damage or growth inhibition, even low-intensity can cause increases in hydrogen ion concentration inside the cytoplasm and disorganization of membrane integrity. For example, Ranalli et al. (2002) have found low-intensity electric current (0.05 and 0.5 mA cm^{-2}; 48 h) induced grown inhibition in *Saccharomyces* species. Lustrato et al. (2006) demonstrated that electric current (1 mA cm^{-2}; 16 d), induced changes

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to the concentration of ATP in *S. cerevisiae*, due to changes in the electrochemical membrane potential which affect the membrane transport and process energy transfer. Assays with *E. coli* showed that low-intensity electric current with graphite electrodes (0.6 and 1.3 mA cm^{-2}; 72 h) provoked no significant changes to enzymatic activities and growth, but at 2.6 and 5.3 mA cm^{-2} inhibition in both was observed. *Bacillus cereus* was not inhibited by the same level of electric current, and at 5.3 mA cm^{-2}; 72 h, growth was stimulated (Valle et al., 2007).

In this study, degradation of hexadecane and biomass production by *Aspergillus niger* was investigated when exposed to low-intensity electric current in a model system consisting of an electrochemical cell packed with an inert support. ATP production was evaluated in order to explain metabolic changes induced by electric current.

2. Methods

2.1. Preparation of inert support and substrate application

Perlite (Dicalite, Mexico; particle size 1.19–1.68 mm), used as inert support, was washed with hot water and dried at room temperature. The support was impregnated with hexadecane (HXD, Sigma) 180 mg, dissolved in 1.9 mL hexane (analytical grade; J.T. Baker, Mexico) per g of dry inert support. Hexane was evaporated in a laminar flow hood for 24 h at room temperature. Since our inert support holds water up to 3.0 times its weight, support moisture was adjusted up 75% with the liquid culture medium (2.3 mL for g dry inert support).

2.2. Microorganism and culture medium

A. niger ATCC 9642 was incubated in potato dextrose agar (PDA; *Bioxon*, Mexico) medium for 7 days (30 °C) and preserved by placing three cubes of PDA with mycelia into cryotubes containing 15% (v v^{-1}) glycerol and storage at −4 °C. The strain was spread again over PDA every 2 months. For growth and degradation studies, spores were harvested with sterile 0.05% water solution of Tween 80 from 7 days old plate cultures. Per g of dry support, 2.0×10^7 spores and 2.3 mL of liquid culture medium (LCM) were added. The medium was composed of (g L^{-1}), 21.2, $NaNO_3$; 3, KH_2PO_4; 0.9, $MgSO_4$; 3, KCl, and 6 mL L^{-1} of a trace element solution containing (g L^{-1}), 0.100, $FeSO_4{\cdot}7H_2O$; 0.015, $CuSO_4{\cdot}5H_2O$; 0.161, $ZnSO_4{\cdot}7H_2O$; 0.008, $MnSO_4{\cdot}7H_2O$ (Volke Sepúlveda et al., 2006). The pH of the medium was adjusted to 5 with 0.1 mol L^{-1}; H_3PO_4 and the medium was autoclaved at 120 °C for 15 min.

2.3. Electrochemical cell

The electrochemical cell (EC) 18 cm long and had a diameter of 6.5 cm. It was made of acrylic glass (poly(methyl methacrylate), 450 mL) and provided with two compartments, each containing 20 mL of electrolyte (0.1 mol L^{-1}; KH_2PO_4) and two electrodes in titanium ruthenium-oxide coated setting with 14.13 cm^2 as contact surface. Electric current was applied at up to 0.42 mA cm^{-2}, corresponding to a 6 V cell potential. Fig. 1 shows the experimental set up of the EC.

2.4. Culture conditions with and without electric current

The EC was packed with 15 g of dry inert support previously impregnated with HXD and the spore suspension and LCM were added. The packed EC was connected to a respirometer (Micro-Oxymax; model System Sample Pump; Columbus Instruments, USA). The O_2 consumption and CO_2 production were recorded on-line during 12 days culture period. After 114 h (30 °C and constant ventilation) the electric current was applied for a 24 h period by using a potentiostat–galvanostat (EG&G; PARC 173, USA), then the electric current was disconnected. In order to measure response variables, EC was divided into three equidistant longitudinal sections between anode and cathode (4 cm per section; see Fig. 1). The following response variables were measured in triplicate for each section: pH, HXD consumption, and the production of biomass and water-soluble metabolites. Measurements were made on cultures with and without exposure to electric current on 0, 4.5, 5.5, 8 and 12 days. At each time point, the whole EC was sacrificed, and for the subsequent samplings a newly prepared culture was used. CO_2 production and O_2 consumption were evaluated through the whole EC.

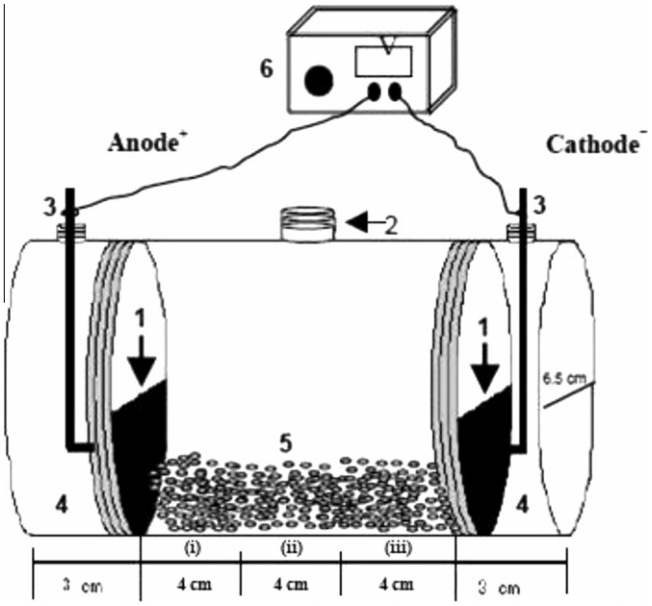

Fig. 1. Electrochemical cell model. (1) Titanium ruthenium-oxide coated electrodes; (2) hermetic valve connected to respirometer for on-line CO_2 and O_2 monitoring; (3) electrolytic solution inlet; (4) reservoir for electrolytic solution; (5) inoculated inert support packed section, and (6) power supply. EC was divided into three equidistant longitudinal sections between anode and cathode (~4 cm per section). (i) Anodic section; (ii) middle section; and (iii) cathodic section.

2.5. Culture under different pH conditions

To distinguish between effects of electric current and pH, experiments were performed without electric current as follows in three 500-mL Wheaton bottles, connected to the respirometer, and containing 15 g of dry inert support impregnated with HXD, growth medium and spore inocula. The bottles were incubated at 30 °C (constant ventilation) for 114 h. Then the pH was adjusted in a gradual manner, *i.e.* all through the next 4 h, until the target pH remained constant, simulating conditions of those experiments with electric current. In order to simulate the middle and cathodic section of the EC with electric current, the pH of the content of two Wheaton bottles was adjusted to 6.4 and 9.1 with 0.1 mol L^{-1} Na_2CO_3, respectively. The third bottle was used to simulate the behavior of the anodic section, and the pH was adjusted to 2.2 with 0.1 mol L^{-1}; H_3PO_4. Biomass production and HXD consumption were measured on 0, 4.5, 5.5, 8 and 12 days. At each time point, the content of the entire bottle was sacrificed, and for the subsequent samplings, newly prepared cultures were used. Analyses were performed in triplicate.

2.6. Biomass production

Biomass was quantified through soluble protein determination with the Lowry (1951) method. In brief, 0.5 g dry samples were ground with liquid nitrogen by mortar and pestle, suspended in 10 mL of 0.5 N NaOH and boiled in a water bath for 10 min. Soluble protein was quantified in the hydrolysed samples with a commercial kit (Bio-Rad Dc protein Assay Kit). A calibration curve was obtained from biomass of *A. niger* grown in 250-mL shaken flasks (50-mL of liquid medium; 72 h). The biomass was vacuum filtered with Whatman 42 paper and ground with liquid nitrogen. Soluble protein was determined spectrophotometrically with a Varian model Cary 50; Australia.

2.7. Determination of residual HXD

Residual HXD was extracted from 1 g of dry sample by using the Soxhlet method US EPA 3540 (US EPA, 1996). The extracted HXD was solubilized in hexane and quantified by gas chromatography (Varian: model 3900; USA) using an Alltech 16367 column (15 m × 0.25 mm) and helium as carrier (30 mL min^{-1}; 40 psi). The oven was heated at 120 °C (30 °C min^{-1}) then the temperature was raised to 120–150 °C (10 °C min^{-1}) and up to 150–170 °C (15 °C min^{-1}); the sample volume injected was 0.002 mL.

2.8. Determination of pH and water-soluble metabolites

One-gram samples of dry inert support were added to 10 mL of distilled water and gently stirred for 5 min. The aqueous fraction was used to determine pH (Conductronic pH 20) and water-soluble metabolites were measured as total organic carbon (TOC) using a TOC analyzer (Shimadzu: model 5000A; Australia).

2.9. Determination of carbon balances and yields

All experimental data (biomass, TOC, HXD, CO_2 and O_2) used to determine the carbon balances and yields were expressed as accumulated averaged values for all the three EC sections (after 12 days of culture). From our experimental values the following yields: biomass to substrate ($Y_{X/HXD}$), oxygen to substrate ($Y_{O_2/HXD}$), and carbon dioxide to substrate ($Y_{CO_2/HXD}$), were calculated using stoichiometrical balances based on one mole of C-atom derived from HXD as the only source of carbon and energy (chemical composition: $CH_{2.125}$); whereas a chemical composition of biomass for *A. niger* $CH_{1.72}O_{0.55}N_{0.17}$ (Nielsen et al., 2003) was included as:

Carbon source	Nitrogen source	Oxygen		Biomass	Water-soluble metabolites (TOC)	Carbon dioxide	Water
$CH_{2.125}$	X1 NO_3	X2 O_2	→	X3 $CH_{1.72}O_{0.55}N_{0.17}$	X4 $CH_2O_{0.125}$	X5 CO_2	H_2O

In such a theoretical assumption, four of the six stoichiometric parameters were experimentally known: consumed oxygen (X2), biomass production (X3), water-soluble metabolites (X4), and produced carbon dioxide (X5). The rest of the parameters were simultaneously solved by algebraic methods for all sampling times.

2.10. Measurement of total ATP content

Cell-free extracts were obtained by grinding 1 g of fresh sample (inert support included) in liquid nitrogen. The material was suspended in 5 mL of phosphate buffer (pH 7) and cells were broken by sonication (Sonicor Instrument; model SC-100; Copiague, NY) at 4 °C; 5 min. Cell debris was removed by centrifugation (18,000*g*; 4 °C; 25 min), and the cell-free extracts were used to determine the soluble protein by Lowry (1951) method (Bio-Rad Dc protein Assay Kit). ATP content was determined according to Tate and Jenkinson (1982) with some modifications. In brief, a commercial kit (ApoGlow® Assay Kit; Lonza Rockland, USA) was used and aliquots (0.08 mL) of each sample from cell-free extracts were placed in a 96-well plate, and 0.1 mL of NRR reagent was added. The mixture was gently stirred, incubated for 5 min at room temperature, and 0.02 mL of NMR reagent (luciferine–luciferase) was added. The mixture was place in a luminometer counting chamber (Multimode Detector; model DTX 880; Beckman Coulter) and integrated reading were taken immediately (1 s) and 10 min later, a second 1-s integrated reading was taken. Finally, 0.02 mL of ADP-CR reagent was added, the 96-well plate was incubated 5 min at room temperature, and a third 1-s integrated reading was taken. The luminescence, expressed in digital relative luminescence units (RLU), was then converted to ATP content according to the calibration curve previously obtained from pure standards.

2.11. Statistical analyses

Data analyses were carried out by using NCSS-2000, version 2001 (Copyright 2001 by Jerry Hintze). Analysis of variance (ANOVA) was performed by comparing tests with a $\alpha < 0.05$.

3. Results and discussion

3.1. Exposure time and electric current selection

In order to find a non-lethal exposure time and electric current intensity we assayed 8 and 19 mA corresponding to potential cell (anode–perlite–anode) of 6 and 20 V, respectively were tested. Cultures were continuously exposed during 12 days. CO_2 production was used as the response variable to decide if exposure time or electric current were lethal and was monitored on-line. In all cases lethal effects, neither CO_2 production nor spores germination, on *A. niger* were observed. For further experiments, excessive exposure times and sporulating forms were avoided. Therefore, after several experiments at different exposure times initiated once spore germination was over, (114 h) and evaluating CO_2 production and HXD degradation we selected the exposure time to be 24 h, and 8 mA as current intensity. When lower exposure time and current were applied to *A. niger* mycelium CO_2 production and cell grow were observed.

3.2. Biomass production and pH effect

Biomass quantification was performed to determine the effect of electric current on fungal growth. Fig. 2a shows the kinetics of biomass produced in the three different EC sections with and without electric current. After applying electric current, *A. niger* growth was considerably diminished throughout the entire EC. Significantly lower levels for biomass (even four times lower) with respect to the EC without current (199 mg per g of dry inert support), were observed. After cessation of the electric current (24 h later), biomass increased in all the three EC sections; however, the total biomass produced was lower at the end of culture than that in the EC without electric current. In both anodic and

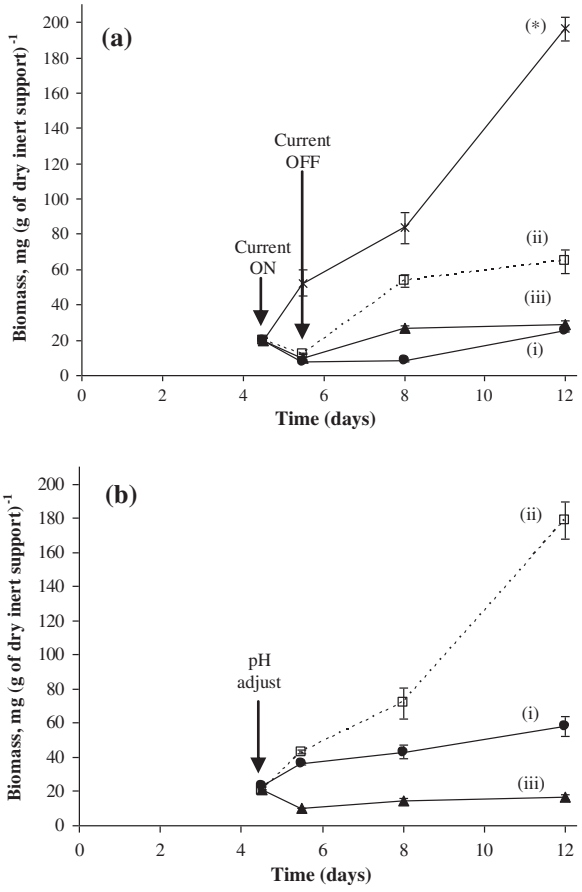

Fig. 2. Kinetics of biomass production: (a) experiments with and without electric current. (b) experiments adjusting pH. (a) Symbols: (∗) –∗–, control (without current); (i) –•–, anodic section, (ii) –□–, middle section; (iii) –▲–, cathodic section. (b) Symbols: (i) –•–, pH 2.2, simulating anodic section; (ii) –□–, pH 6.3, simulating middle section; (iii) –▲–, pH 9.1, simulating cathodic section. Error bars represent standard deviations of three replicates for all assays..

cathodic section, biomass production was similar 25 and 29 mg per g of dry inert support, respectively (Fig. 2ai and aiii), whereas in the middle section, higher biomass production of 65 mg per g of dry inert support was observed after 12 days of culture (Fig. 2aii).

Such growth inhibiting could have been due to temperature-induced lysis of (Palaniappan et al., 1992), production of toxic substances (Guillou and El Murr, 2002), or pH variation (Wenyan et al., 2005). Temperature effects are unlikely since the temperature never exceeded 27 °C. The production of toxic substances was also unlikely since electrodes made with a titanium ruthenium-oxide coating were used. Nevertheless, changes in pH could have occurred at the electrodes (Xin et al., 2010). As our measurements showed, significant pH gradients were found varying from: 2.2 (anodic section), 5.5 (middle section) and 9.1 (cathodic section). During experiments without electric current, pH only increased one unit as compared to the initial value (from 5 to 6.3), because of metabolic reactions during hexadecane degradation. The Fig. 2b shows the kinetics of biomass production in experiments without electric current, but at pH levels similar to those in each EC section. At the end of culture, the biomass produced was two and three times higher in the anodic and middle EC section, respectively (Fig. 2bi and bii), than that observed in the respective sections with electric current (Fig. 2ai and aii). In contrast, the biomass observed in both cathodic section and pH simulated cathodic section (Fig. 2aiii and biii) was not significantly different. Therefore, our results demonstrated that *A. niger* grown inhibition was mainly due to electric current effects and to pH gradients to a lesser extent.

3.3. Hexadecane degradation and pH effect

Fig. 3a shows the kinetics of HXD degradation, with and without electric current. Before electric current was applied (4.5 d), the average HXD degradation was 38%. At the end of culture, without electric current, degradation was 81 ± 1.2% (as average of the cell sections). Despite the fact that growth was inhibited, degradation levels were significantly higher, on average in the three EC sections (96 ± 1.4%), than those observed without current. With electric current, HXD was almost depleted in the three EC sections in less than 8 days of culture.

When biodegradation rates of HXD were compared with and without electric current, we observed a significantly higher rate of 27 ± 2 mg of HXD per g of dry inert support per day on average for the three cell sections, with respect to the EC without current 17 ± 4 mg of HXD per g of dry inert support per day. Fig. 3b shows the kinetics of HXD degradation in experiments without electric current under pH conditions mimicking those in each EC section. In all cases, only a low level of degradation of 40%, 22% and 27% at pH 9.1, 6.3 and 2.2 respectively was observed. When results obtained with the pH-adjusted cultures were compared with those

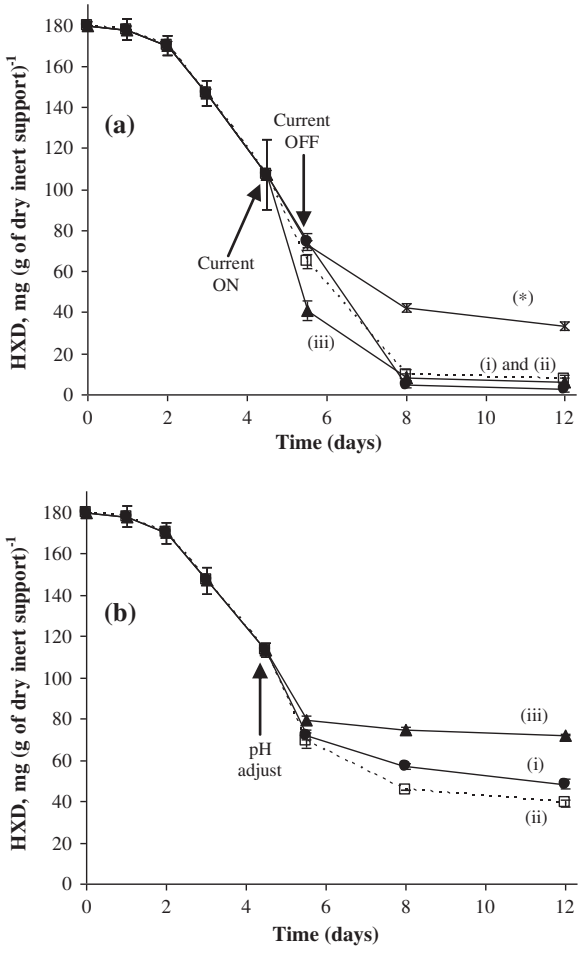

Fig. 3. Kinetics of hexadecane degradation: (a) Experiments with and without electric current. (b) Experiments adjusting pH. (a) Symbols: (∗) –∗–, control (without current); (i) –•–, anodic section, (ii) –□–, middle section; (iii) –▲–, cathodic section. (b) Symbols; (i) –•–, pH 2.2, simulating anodic section; (ii) –□–, pH 6.3, simulating middle section; (iii) –▲–, pH 9.1, simulating cathodic section. Error bars represent standard deviations of three replicates for all assays.

obtained with cultures exposed to electric current, several aspects emerged:

(i) When pH-was adjusted to 9.1, the growth was up to 17 mg of biomass (g of inert support)$^{-1}$ and HXD degradation was the lowest attained (60%; after 12 d); while at the cathodic section (which generated the same pH value) biomass was as higher as 28.9 mg (g of inert support)$^{-1}$ and HXD degradation was 95% after 8 days of culture.
(ii) When pH-was adjusted to 6.3, the growth was 178 mg of biomass (g of inert support)$^{-1}$ and HXD degradation was 78% after 12 days of culture; while in the middle section with electric current, biomass was 65 mg (g of inert support)$^{-1}$ and HXD degradation was 95% after 8 days.
(iii) Finally, when pH-was adjusted to 2.2, the growth increased twice as compared to anodic section where HXD degradation was 73% after 12 d; while at the anodic HXD degradation was 97% after 8 days.

During electric current application, without inoculum, no HXD degradation was observed. Our results demonstrate that *A. niger* is able to degrade high levels of HXD in a short period of time when electric current is applied, suggesting that this effect is enhanced by the electric current applied to *A. niger*, and does not depend on the pH generated. Our results agree with data reported by Shin et al. (2002) who reported that electric current (generating a voltage of 1.5 V) between 15 and 78 h to *Clostridium thermocellum* (ATCC 35609) culture, increased ethanol production up to 52% compared to the control (without electric current). Xin-gang et al. (2006) demonstrated that electric current (3 V; 21 h, in wastewater sludges), increased phenol degradation up to 33%, compared to control (without electric current). Jackman et al. (1999) demonstrated that under acid conditions and in the presence of an electric current (20 mA cm^{-2}; 80 h), growth of acidophilic bacteria declined. All of these authors concluded that the observed effects depended on the electric current that had passed through the cell suspensions. Golzio et al. (2004) demonstrated that cells can be made temporarily permeable by electric fields. Such results suggest that HXD is probably allowed to be transported into the cell faster with electric current than without.

3.4. CO_2 production and O_2 consumption

Since O_2 consumption can be associated with the initial biotransformation of the carbon source (HXD), and CO_2 production is related to mineralization of the same source, these activities were continuously measured on-line during the 12 days of cultivation. Fig. 4 shows accumulated CO_2 production and O_2 consumption kinetics.

In EC with electric current, O_2 consumption was evidently higher (up to fourfold) compared to control EC (without electric current) at the end of culture (Fig. 4a). Before applying electric current, there were no statistical differences between both conditions; however, as electric current (0.42 mA cm^{-2}, during 24 h) passed through the EC O_2 consumption increased (Fig. 4ai). Moreover, a higher positive-slope was maintained during and after electric current stimulation as compared to control (see Fig. 4ai and aii). At the end of the experiments, 586 ± 58 mg of O_2 per g of dry inert support was consumed in EC with electric current and 151 ± 50 mg of O_2 per g of dry inert support for the control EC. Fig. 4b shows CO_2 production during 12 days of culture. A substantial increase of up to threefold was observed in EC with electric current as compared to the control. There were no differences in CO_2 production during the first 4.5 days before electric current stimulation. After day 6, differences in CO_2 production were evident: 405 ± 75 and 114 ± 04 mg of CO_2 per g of dry inert support

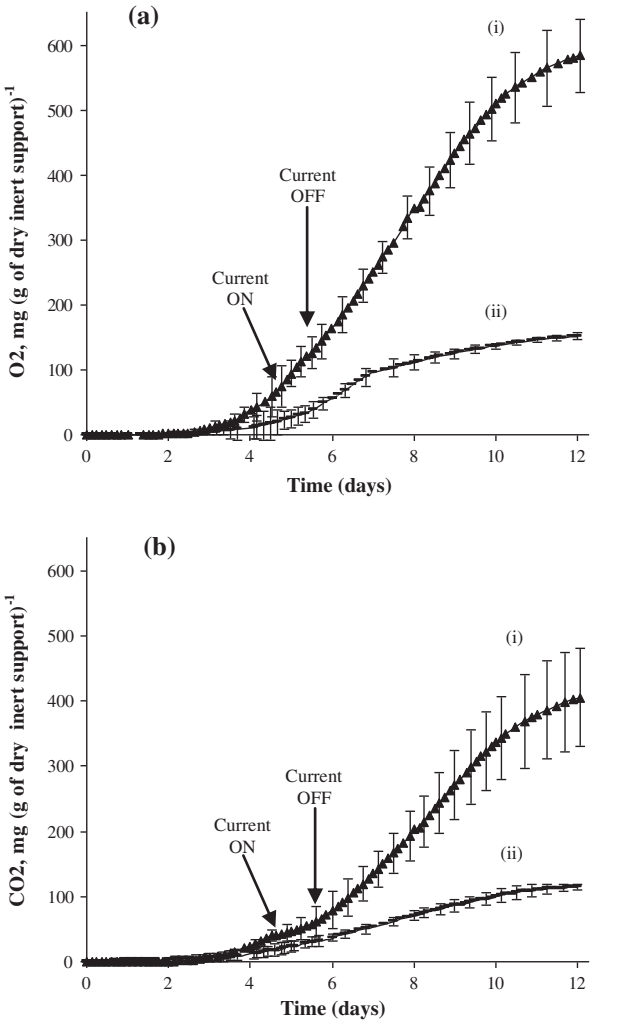

Fig. 4. Accumulated oxygen consumption (a) and accumulated carbon dioxide production (b), during 12 days of culture, in the presence (i), and the absence (ii) of electric current. Error bars represent standard deviations of three replicates for all assays.

for EC with electric current and control, (Fig. 4bi and bii), respectively. These results suggest that electric stimulation induced higher substrate oxidation and mineralization, as compared to the same culture in the absence of electric current.

3.5. Carbon balances and yields

Fig. 5 shows recovered carbon distributed among all carbon-species, during 12 days of culture in EC with or without electric current. Original carbon balances distributions were strongly modified by electric current. At the end of the culture, in the EC control experiments (Fig. 5a), biomass production was predominantly (76 ± 0.09%) associated to low CO_2 yields (25 ± 0.04%) and minimal formation of soluble compounds (5 ± 0.01%). On the contrary, in current-treated EC (Fig. 5b), biomass production was as low as 13 ± 0.01%, associated with high CO_2 yields (74 ± 0.14%) and also minimal formation of soluble compounds, only 4 ± 0.01% of the total carbon distribution.

Table 1 shows yields with and without electric current, after 12 days of culture. In control EC, high $Y_{X/HXD}$ values were observed contrasting with those with electric stimulation. The current stimulation was able to increase $Y_{O_2/HXD}$ values by more than three times, compared to control. These results suggest that O_2 con-

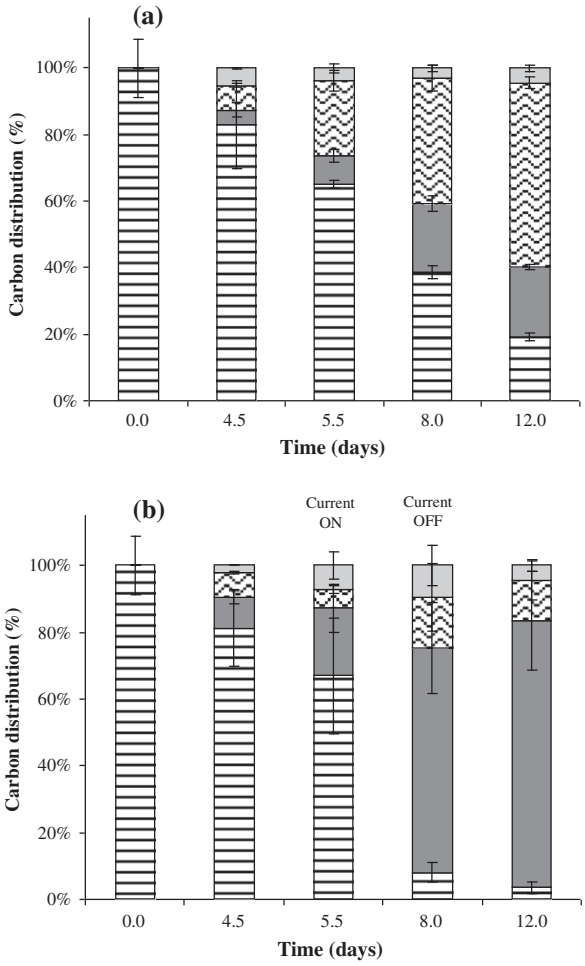

Fig. 5. Carbon balances for hexadecane biodegradation by *A. niger*, after 12 days of culture. (a) Control without electric current. (b) With electric current. Symbols: C-HDX; C-CO_2; C-biomass and C-TOC. Error bars represent standard deviations of three replicates for all assays.

sumption in control EC was mainly used to produce biomass, whereas O_2 consumption with electric current was mainly employed to mineralize HXD, as shown by the $Y_{CO_2/HXD}$ yield. Our results showed changes in the metabolism of *A. niger* in the EC with electric current. The carbon balances without electric current suggested a more assimilative metabolic activity, which completely changed to a non-assimilative, when electric current was applied.

3.6. Total ATP content

Total ATP concentration was evaluated as an indicator of metabolic activity during the treatment with electric current. The intracellular ATP content before and after electric current application is shown in Fig. 6. Without current the total ATP content decreased 61% by the end of culture (12 d) as compared with initial time (4.5 d). This result suggests that ATP was employed to increase biomass and HXD degradation. In contrast, total ATP content mark-

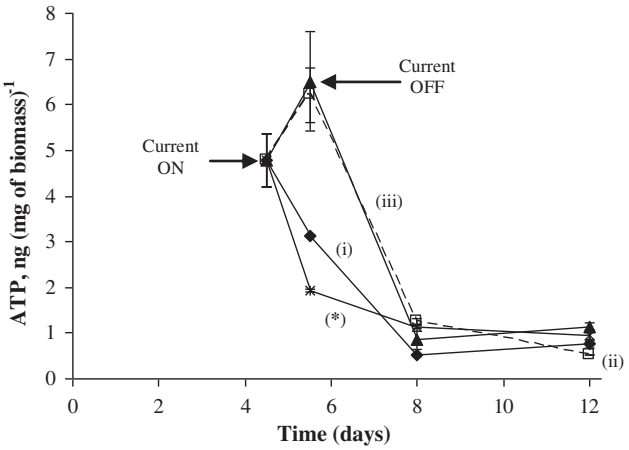

Fig. 6. Total ATP content in *Aspergillus niger*, as a function of time, with and without electric current. Symbols: (∗) −∗−, control (without current); (i) −•−, anodic section, (ii) –□–, middle section; (iii) −▲−, cathodic section. Error bars represent standard deviations of three replicates for all assays.

edly increased after applying electric current (at 5.5 d). In the cathodic and middle sections there were no differences in total ATP content during current application. The anodic section showed low total ATP content compared to the cathodic and middle sections; however, it was 1.5-fold higher than that of the control.

In the cathodic and middle sections, total ATP content was, respectively, 34% and 28% higher than that found before electric current treatment. This effect is correlated to the increase in HXD degradation and inhibition of biomass production observed in the same EC section and same period of time. These results suggest that the electric current stimulate metabolic activity, probably at the mitochondrial level as suggested by Vajrala et al. (2008) who demonstrated, with three-membrane model of cells containing mitochondria, that low (1 V cm^{-1}) electric current can induce mitochondrial modifications.

Our results are in line with those reported by Valle et al. (2007), who observed stimulation in the total ATP content after low-intensity current application to *B. cereus*. Lustrato et al. (2006) and Guillou et al. (2003) also observed that total ATP content increased after low electric current application in *S. cerevisiae*.

In the anodic section, contrary to the cathodic and middle sections, total ATP content decreased by 34%, in the same way as experiments without current, but with low biomass production. This effect can be explained by the effects of the low pH of 2.2 (Escoffre et al., 2007). Total ATP values reinforce the idea that the effect of electric current modifies the metabolic activity in *A. niger*.

4. Conclusions

Low-intensity electric current application to *A. niger* stimulated catabolic (non-assimilative) metabolism, despite pH changes generated by the electric current. This effect was linked to decreased biomass production, transient increase in total ATP and to increased HXD degradation as compared to the control without electric current. *A. niger* is an important microorganisms for

Table 1
Biomass to HXD yield ($Y_{X/HXD}$), O_2 to HXD yield ($Y_{O_2/HXD}$), and CO_2 to HXD yield ($Y_{CO_2/HXD}$) for *Aspergillus niger*, with and without electric current, after 12 days of culture.

	$Y_{X/HXD}$[a], mol C-biomass (mol consumed C-HXD)$^{-1}$	$Y_{O_2/HXD}$[a], mol O_2 (mol consumed C-HXD)$^{-1}$	$Y_{CO_2/HXD}$[a], mol C-CO_2 (mol consumed C-HXD)$^{-1}$
With current	0.13 ± 0.01	1.48 ± 0.14	0.74 ± 0.14
Without current (control)	0.76 ± 0.09	0.46 ± 0.07	0.25 ± 0.04

[a] Averaged and standard deviation values of three replicates.

biotechnological purposes and our data suggest the possibility of manipulating its metabolism by using electric current.

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References

Bawcom, D.W., Thompson, L.D., Miller, M.F., Ramsey, C.B., 1995. Reduction of microorganisms of beef surfaces utilizing electricity. J. Food Protect. 58, 35–38.

Escoffre, J.M., Dean, D., Hubert, M., Rols, M.P., Favard, C., 2007. Membrane perturbations by an external electric field: a mechanism to permit molecular uptake. Eur. Biophys. J. 36, 973–983.

Ganeva, V., Galutzov, B., Teissie, J., 2002. Electroinduced release of invertase from *Saccharomyces cerevisiae*. Biotechnol. Lett. 24, 1853–1856.

Golzio, M., Rols, M.P., Teissié, J., 2004. In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expresión. Methods 33, 126–135.

Guillou, S., Besnard, V., El Murr, N., Federighi, M., 2003. Viability of *Saccharomyces cerevisiae* cells exposed to low amperage electrolysis as assessed by staining procedure and ATP content. Int. J. Food Microbiol. 88, 85–89.

Guillou, S., El Murr, N., 2002. Inactivation of *Saccharomyces cerevisiae* in solution by low-amperage electric treatment. J. Appl. Microbiol. 92, 860–865.

Hyo-Sang, L., Lee, K., 2001. Bioremediation of diesel-contaminated soil by bacterial cells transported by electrokinetics. J. Microbiol. Biotechnol. 11, 1038–1045.

Jackman, A.S., Maini, G., Sharman, K.A., Knowles, J.C., 1999. The effects of direct electric current on the viability and metabolism of acidophilic bacteria. Enzyme Microb. Technol. 24, 316–324.

Lear, G., Harbottle, M.J., van der Gast, C.J., Jackman, S.A., Knowles, C.J., Sills, G., Thompson, I.P., 2004. The effect of electrokinetics on soil microbial communities. Soil Biol. Biochem. 36, 1751–1760.

Lowry, O.H., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Lustrato, G., Alfano, G., Belli, C., Grazia, L., Iorizzo, M., Ranalli, G., 2006. Scaling-up in industrial winemaking using low electric current as an alternative to sulfur dioxide addition. J. Appl. Microbiol. 101, 682–690.

Nielsen, J., Villadsen, J., Lidén, G., 2003. Bioreaction Engineering Principles, second ed. Kluwer Acedemic/Plenum Publishers, New York. p. 56.

Palaniappan, S., Sastry, S.K., Richter, E.R., 1992. Effect of electroconductive heat treatment and electrical pretreatment on thermal death kinetics of selected microorganisms. Biotechnol. Bioeng. 39, 225–232.

Ranalli, G., Iorizzo, M., Lustrato, G., Zanardini, E., Grazia, L., 2002. Effects of low electric treatment on yeast microflora. J. Appl. Microbiol. 93, 877–883.

Shin, H.S., Zeikus, J.G., Jain, M.K., 2002. Electrically enhanced ethanol fermentation by *Clostridium thermocellum* and *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 58, 476–481.

Tate, K.R., Jenkinson, D.S., 1982. Adenosine triphosphate measurement in soil: an improved method. Soil Biol. Biochem. 14, 331–335.

Teissié, J., Eynard, N., Vernhes, M.C., Bénichou, A., Ganeva, V., Galutzov, B., Cabanes, P.A., 2002. Recent biotechnological developments of electropulsation, a prospective review. Bioelectrochemistry 55, 107–112.

Thrash, J.C., Coates, J.D., 2008. Review: direct and indirect electrical stimulation of microbial metabolism. Environ. Sci. Technol. 42, 3921–3931.

US EPA, 1996. ASW-846, Test Methods for Evaluating Solid Wastes Physical/Chemical Methods, EPA methods 3540 and 8100, third ed. Environmental Protection Agency, Washington DC.

Vajrala, V., Claycomb, J.R., Sanabria, H., Miller Jr., J.H., 2008. Effects of oscillatory electric fields on internal membranes: an analytical model. Biophys. J. 94, 2043–2052.

Valle, A., Zanardini, E., Abbruscato, P., Argenzio, P., Lustrato, G., Ranalli, G., Sorlini, C., 2007. Effects of low electric current (LEC) treatment on pure bacterial cultures. J. Appl. Microbiol. 103, 1376–1385.

Virkutyte, J., Sillanpaa, M., Latostenmaa, P., 2002. Electrokinetic soil remediation critical overview. Sci. Environ. 289, 97–121.

Volke Sepúlveda, T.L., Gutiérrez-Rojas, M., Favela-Torres, E., 2006. Biodegradation of high concentrations of hexadecane by *Aspergillus niger* in a solid-state system: Kinitec analysis. Bioresour. Technol. 97, 1583–1591.

Wenyan, L., Jiuhui, Q., Libin, Ch., Huijuan, L., Pengju, L., 2005. Inactivation of *Microcystis aeruginosa* by continuous electrochemical cycling process in tube using Ti/RuO_2 electrodes. Environ. Sci. Technol. 39, 4633–4639.

Xin, L., Yunguo, L., Guangming, Z., Yile, N., Xin, X., Weihua, X., Wenbing, X., Yun, Z., Jiadong, L., 2010. Direct current stimulation of *Thiobacillus ferrooxidans* bacterial metabolism in a bioelectrical reactor without cation-specific membrane. Bioresour Technol. 101, 6035–6038.

Xin-gang, L., Tao, W., Jin-sheng, S., Xin, H., Xiao-song, K., 2006. Biodegradation of high concentration phenol containing heavy metal ions by functional biofilm in bioelectro-reactor. J. Environ. Sci. 18, 639–643.

Zanardini, E., Valle, A., Gigliotti, C., Papagno, G., Ranalli, G., Sorlini, C., 2002. Laboratory-scale trial of electrolytic treatment on industrial wastewaters: microbiological aspects. J. Environ. Sci. Health Pt A Toxic/Hazard. Subst. Environ. Eng. 37, 1463–1481.