Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

Vol.2. No.4: 27-37. July to December. 2013.

Evaluation of microbial enzyme activities in Sontecomapan lagoon, Veracruz, Mexico.

Evaluación de actividades enzimáticas microbianas en la laguna de Sontecomapan, Veracruz, México.

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ABSTRACT

The estimation of enzymatic activities in aquatic systems constitutes an instrument of evaluation of the functional diversity of the microbiota, the carbon flows, the nutriments recycling rate and the ecosystem health level. The microbial hydrolytic activity is fundamental to understand the processes of new and regenerated production in aquatic ecosystems; nevertheless, the techniques for determining this activity have been mainly developed on agricultural grounds. Reason why in this study the techniques were adapted with the aim to determine *ex situ* the dehydrogenase (DHA), cellulase (CA), acid and alkaline phosphatase (AcFA and AlFA) and chitinase (QA) microbial enzymatic activities, in bottom water and superficial sediments of Sontecomapan lagoon, Veracruz, at north winds and dry seasons. The capacity of bacterioplankton and bacteriobenthos to produce extracellular enzymes (cellulase, phosphatase, chitinase, amylase, DNAase, lecitinase and esculinase) was also determined, and the variations of the physical and chemical parameters in the different sampling stations at two climatic times were measured. The principal components analysis showed that the greater microbial hydrolytic activity appeared in the sediments, only the DHA and the FA were higher in bottom water in dry season. In the same way, the bacterial extracellular enzyme production was greater in the bottom water. The statistical analysis showed that low oxygen and Eh values lead to a high expression of the DHA and FA (acid and alkaline), whereas the QA was inhibited by an alkaline pH and high salinities. Both microbial and bacterial hydrolytic activity in superficial sediments showed a high correlation with the organic matter concentration.

Key words: Bottom water, hydrolysis, microbenthos, microplankton, sediments.

RESUMEN

La estimación de actividades enzimáticas en sistemas acuáticos constituye un instrumento de evaluación de la diversidad funcional de la microbiota, los flujos de carbono, la tasa de reciclamiento de nutrimentos y del estado de salud del ecosistema. La actividad hidrolítica microbiana es fundamental para entender los procesos de producción nueva y regenerada en ecosistemas acuáticos; sin embargo, las técnicas para determinar esta actividad han sido desarrolladas sobre todo en suelos agrícolas, por lo que en este estudio se adecuaron las técnicas para determinar *ex situ* las actividades enzimáticas microbianas: deshidrogenasa (ADH), celulasa (AC), fosfatasa ácida y alcalina (AFAc y AFAl) y quitinasa (AQ) en agua de fondo y sedimentos superficiales de la laguna de Sontecomapan, Veracruz, en época de nortes y secas. Se determinó, igualmente, la capacidad del

Receive: September 5th, 2013. Accepted: November 30th, 2013. Published: December 1st, 2013

Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

Vol.2. No.4: 27-37. July to December. 2013.

bacterioplancton y bacteriobentos de producir enzimas extracelulares (celulasa, fosfatasa, qutinasa, amilasa, ADNasa, lecitinasa y esculinasa) y se midieron las variaciones de los parámetros físicos y químicos en las diferentes estaciones de muestreo en las épocas climáticas antes mencionadas. El análisis de componentes principales mostró que la mayor actividad hidrolítica microbiana se presentó en los sedimentos, sólo la ADH y AF fueron mayores en agua de fondo en temporada de secas. De igual manera, la producción de enzimas extracelulares bacterianas fue mayor en el agua de fondo. El análisis estadístico mostró que bajos valores de oxígeno y de Eh promovieron una alta expresión de la ADH, AFAc y AFAl, mientras que la AQ fue inhibida por el pH alcalino y las altas salinidades. Tanto la actividad hidrolítica microbiana como bacteriana en los sedimentos superficiales, mostraron una alta correlación con la concentración de la materia orgánica.

Palabras clave: Agua de fondo, hidrólisis, microbentos, microplancton, sedimentos.

INTRODUCTION

In all ecosystems, the microorganisms (bacteria, fungi, protists-seaweed and protozoans) are most abundant and transfer a great amount of matter and energy. The rates of nutrients recycling (C, N, P) are controlled in direct form by means of hydrolytic degradation of organic compounds, or indirect form modifying the retention or movement of the nutrients (p.e. modifications in the redox potential) (Alvarez, 2005). The biogeochemical balances in coastal ecosystems allow evaluate the charges, the flows and the destiny of the nutriments, as well as the net metabolism of the ecosystem. The decomposition of the organic matter (OM) deposited in the sediments enriches the interstitial water with soluble nitrogen (NH_4^+, NO_2^- y NO_3^-), phosphorus (HPO_4^{2-}), sulphur (SH^-, SO_4^{2-} y H_2S) and iron forms (Fe^{2+} y Fe^{3+}) (Moran-Villa, 2007). The result of the decomposition of the OM is the liberation and accumulation of soluble monomers, oligomers and macro-molecules, but bacteria, microplankton and microbenthos (microscopic invertebrates, plankton and benthos larvae's) are the main organisms that participate in organic compound mineralization due to the presence of specific exoenzymes which catalyze high molecular weight compounds hydrolysis (Curticapean and Dragan 2007). The enzymatic activity constitutes therefore a critical and fundamental step in the processing of the detritus (Alvarez 2005).

The detrital carbon is the main trophic resource of many aquatic systems, its entrance to the trophic network is made through heterotrophic microorganisms whose collective activity can be expressed as particulate organic carbon degradation rates (POC), dissolved organic carbon assimilation (DOC) and microbial biomass production. The enzymatic potential in sediments reflects, by consequence, the activity of the micro biota, the influence of physical, chemical and anthropogenic factors and the intensity of the enzymatic activity (Curticapean and Dragan 2007).

It is very complex to analyze the microbial fraction that takes to end the most OM decomposition and mineralization processes; nevertheless, now, techniques have been developed in order to study the operation *in situ* of these communities (Alvarez 2005). The knowledge of the role played by the microbial communities in soils and sediments can help understanding and maintaining the system operation, since the main function of the microbenthos in the sediments is in its participation in the cycle of nutrients from detritus (Caldwell 2005).

The microbial oxidation of organic substances under aerobic conditions is linked to a chain of electrons transport, connected to the ATP synthesis, and which has the oxygen as a final electrons acceptor and is known as oxidative phosphorylation. In the main way of the electronic transport from the organic substrates to molecular oxygen, four types of redox enzymes take a part; among them, there are pyridin-dependent dehydrogenases, which need NAD or NADP as coenzymes and flavin-dependent dehydrogenases containing FAD or FMN as prosthetic group. Thus, the microorganisms total dehydrogenase activity depends on different dehydrogenases activities and has a fundamental role in the initial stages of the organic matter oxidation.

Knowledge of the microbial hydrolytic activity is fundamental to understand the regenerated processes in aquatic systems; nevertheless, there is very little information on the techniques for

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determining the microbial exoenzymatic activity in aquatic ecosystems (Frutos 2004).

This is an obstacle for the knowledge and the diagnostic of the preservation conditions of aquatic ecosystems with ecological vulnerability problems. It is the reason why one of the objectives of this work was: 1) to adapt the techniques for determining the total *ex situ* microbial hydrolytic activity, through dehydrogenase, cellulase, phosphatase and chitinase enzymes in bottom water and lagoon superficial sediments, and 2) to determine the bacterioplankton and bacteriobenthos hydrolytic potentiality present in Sontecomapan lagoon, Veracruz.

MATERIAL AND METHODS

Study zone

The lagoon of Sontecomapan belongs to the region of basin formed by the San Martín Tuxtla volcano and the Sierra de Santa Martha, and it is located between the parallels 18° ' - 18° 34 'N and meridians 94° 54 ' - 95° 02 'W, south of Veracruz State, southern Gulf of Mexico (Fig. 1). Its area is 8.9 km^2 and it has an important fishing population, areas of culture and some zones of cattle activity. The lagoon has a depth average of 1.5 m. It is connected permanently to the sea through a mouth, presents mixohyalin environments by influence of fresh and marine waters (González et al. 1994).

Sampling

Sediments

The sediment samples for the microbial enzymatic activities measurements was obtained by free diving using 17 cm long polycarbonate manual corers and 4,5 cm inner diameter (i.d.), avoiding to disturb the superficial layer of the sediment. With the help of a piston, the first centimeter of sediment was cut in order to obtain sub-samples of two cubic centimeters using a cut end sterile hypodermic syringe. The samples were preserved in refrigeration, in plastic bags with hermetic seal at low temperature. For the bacterial enzymatic activities analysis, two cubic centimeters of sediment were preserved in sterile bottles containing 18 ml of glycerol to 50% and deep frozen to -21°C until their processing.

In order to obtain the concentration of organic matter and to determine the texture of the sediments, the sediments samples were conserved in hermetic plastic bags at room temperature.

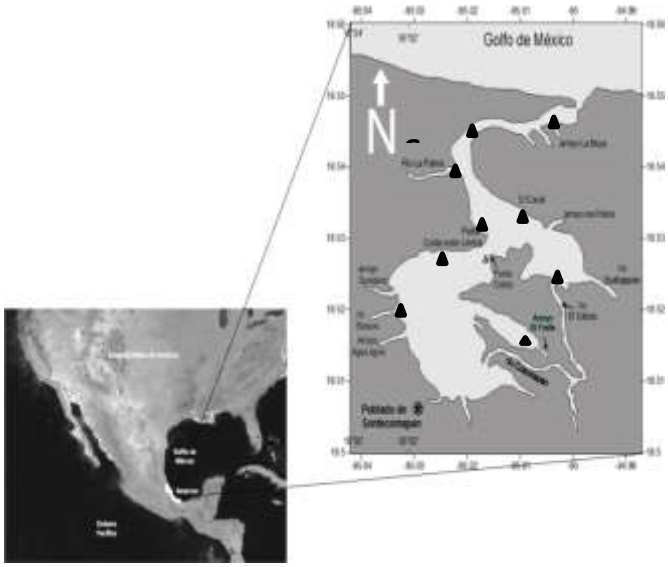

Fig. 1. Location from sampling sites in Sontecomapan lagoon, Veracruz

Water

The bottom water samples for microbial and bacterial enzymatic activities were obtained with a 1L horizontal Van Dorn bottle, and 150 mL from the water sample were fixed in an equal volume of sterile glycerol to 50%, and stored to -20°C until processed in laboratory. For the determination of nitrogen and phosphorous concentration the samples were filtered using Whatman GF/F membranes and preserved to -20°C for its later analysis in laboratory. The interstitial water was obtained from

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the not-disturbed sediment cores using a 5 mm outer diameter (o.d.) and a 10 cm long capillary tube, with a series of millimeter perforations throughout the first centimeter of the capillary tube tip and, the least upper bound connected to a hose connected to a syringe of 50 mL. The water obtained was filtered through a 25 mm diameter GF/F Millipore membrane and stored to -20°C in penicillin type bottles previously gasified with N_2, until it's processing.

Physical and chemical parameters

The percentage of organic matter (OM) was measured by the Walkey and Black titration method (1934), the grain sized classification by the Bouyoucos method (Villegas et al. 1978), the suspended materials (SMA) were calculated according to Strickland and Parsons (1972), and the dissolved oxygen by the Winkler (1998) technique.

The pH and Eh in bottom water were measured with an ATAGO S/Mill-E® potentiometer and an YSI 556 MPS® multiparametric, respectively. In sediment, Eh was measured with the use of a Thermo Orion® 250A+ potentiometer and a 5 mm o.d. Sentek® glass redox minielectrode.

The nitrogenated inorganic substrates concentration was determined by the spectrophotometric methods recommended by Aminot and Chaussepied (1983); the nitrates (N-NO_3-) in bottom water by the method of cadmium reduction. The nitrites (N-NO_2-) and ammonium (N-NH_4^+) concentration by the method of Koroleff (1969). The phosphorus determination (total phosphorus and orthophosphates) in bottom and interstitial water was made with a HACH® spectrophotometer using specific kits for marine water (method of digestion with persulphate 8190 with detection rank from 0,02 to 1,10 mg L^{-1}; method of amino acids 8178 with detection rank from 0,23 to 30 mg L^{-1} respectively).

The nitrate concentration in interstitial water was determined by the method of cadmium reduction using a Hach kit for method 8171 with a range of sensitivity from 0.1 to 10 mg L^{-1}.

Microbial and bacterial enzymatic activities

The method for the dehydrogenase activity (DHA) calculation is based on the valuation of Iodonitrotetrazolium formazan (INTF) formed when 3 gr of sediment or 3 mL of bottom water are incubated with 0,2 ml of 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium (INT) to 0,4%, for 20 hours at 20°C in darkness (Garcia et al., 1993). The standard curve was prepared from an INTF standard solution of 60 µg L^{-1} and a series of concentrations were made from 0 to 60 µg mL^{-1}, by duplicate and measured at a wavelength of 490 nm.

For the cellulose activity evaluation, the method by Somogy-Nelson was used (Nelson, 1994), in which the sugars are oxidized by cupric compounds (Cu^{2+}) in alkaline solution. The reducing sugars are oxidized by copper sulphate (Cu^{2+}) which is reduced to Cu_2O, which forms, at its time, a blue color complex when reacting with the Nelson's reagent (Garcia and Ibañez, 1994). A standard curve of glucose reducing sugars from a 1 mM standard solution, and series of standard concentrations from 0.5 to 10 mM was made by duplicate. The cellulase activity was calculated with a spectrophotometer at a wavelength of 540 nm using 5 gr of sediment dried at room temperature and sifted on 0.2 mm diameter enmeshes or five mL of bottom water (Garcia and Ibañez 1994).

Phosphatases are enzymes that catalyze esters and anhydrides hydrolysis of phosphoric acid. Alkaline and acid phosphatases belong to the group of monoesterphosphate hydrolase, which are two unspecific enzymes that catalyze glycerophosphate hydrolysis; the difference between them is its operating optimal pH. The phosphatase activity estimation is based on the spectrophotometric determination of the p-nitrophenol released when 1 gr of dry sediment or 1 mL of bottom water are incubated at 37 °C during one hour with a buffered dissolution of p-nitrophenylphosphate (pH 6,5 for the acid phosphomonosterase and pH 11 for the alkaline one). The colorimetric method for the p-nitrophenol released measurement is because the alkaline dissolutions of this compound present a yellow color (Tabatabai and Bremer, 1969). The standard curve was prepared from a standard solution of 1000 µg mL^{-1} p-nitrophenol and series of concentrations from 0 to 250 µg mL^{-1} by duplicate.

Receive: September 5th, 2013. Accepted: November 30th, 2013. Published: December 1st, 2013

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ISSN: 2007-5782

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For the chitinase activity, the determination is based on the spectrophotometric valuation (wavelength of 590 nm) of the N-acetylglucosamine formed by enzymatic hydrolysis when a 5 gr of dry sediment sample or 5 mL of bottom water are incubate in the presence of a well-known amount of chitin (10 ml of a suspension at 5% of chitin) (Rodriguez et al. 1983).

Bacterial enzymatic activities

In order to demonstrate the extracellular enzymes production by the heterotrophic bacterioplankton and bacteriobenthos, eight specific organic substrates with ZoBell agar as base medium (BM) (ZoBell 1941) were used. The phosphatase production was observed in the base medium containing 1% of a p-nitrophenylphosphate sodium solution (Sigma) (Tramer, 1952), and the protein hydrolysis by addition of 4 g L^{-1} of gelatin to the BM (Lányi 1987). The amylase production was observed adding 10g L^{-1} starch and the DNA hydrolysis in BBL® DNAase agar (Jeffries et al. 1957). For the esculine, adding 0.1 % of esculine and 0.1 % of ferric salt (Cowan and Steel, 1974). Concerning the long chain carbohydrates, as chitin, by addition of 4 g L^{-1} colloidal chitin; the fatty acid hydrolysis by the production of lecithinase adding to the BM 10% of egg white. The lipases by the production of tweenesterase, adding 10 ml L^{-1} of tween 80 (Lányi 1987), and of the cellulase in liquid BM with a rice paper strip (Angeles-Vázquez 2007). All analyses of microbial enzymes production were realized in quadruplicate.

Statistical analysis

The Principal Components analysis (the PCA) allows us to evaluate different variables simultaneously. The graph of the PCA represents the arrangement of the enzymatic and physico-chemical variables according to the axes `x' and `y', which represent eigen vectors with the greater variance (their eigen vector 1 and 2 respectively). At the same time the categorical seasonal variables of the year, salinity and sampling sites were added. The PCA was made with the function `dudi.pca' using the package `ade4' (Dray et al. 2007) in program R 2.15.3 (R Core Team 2013).

RESULTS AND DISCUSSION

Physico-chemical characterization of the bottom water and superficial sediments

The average temperature did not present a variation between the bottom water and the sediment in both climatic stations; but at droughts time it was greater than at north winds time, when the average depth was smaller (Table 1).

Table 1. Average values ± SD of the values from the physico-chemical parameters measurement in the bottom water (bw) and in the superficial sediments (s) (1 cm depth) from the different sampling stations, and in the two seasons of the year studied.

Parameters	Biotope	North winds	Dry season
Depth (cm)	bw	115.6 ± 37.7	133.6 ± 136.6
Temperature (°C)	bw	23.3 ± 1.5	30.7 ± 2.2
	s	22.2 ± 1.8	30.9 ± 2.6
Salinity (ppm)	bw	14.2 ± 12.2	20.8 ± 8.9
	s	19.3 ± 13.0	7.9 ± 9.4
Oxygen (mg L^{-1})	bw	3.8 ± 0.8	5.6 ± 0.9
pH	bw	9.0 ± 0.8	7.9 ± 0.6
	s	8.9 ± 0.7	8.0 ± 0.4
Eh (mV)	bw	45.3 ± 41.5	95.2 ± 71.3
	s	−35.3 ± 74.5	−50.0 ± 160.0
N-NH_4^+ (mg L^{-1})	bw	0.04 ± 0.04	0.01 ± 0.02
	s	0.05 ± 0.04	0.09 ± 0.05
N-NO_2^- (mg L^{-1})	bw	0.01 ± 0.02	0.002 ± 0.002
	s	0.01 ± 0.01	0.06 ± 0.03
N-NO_3^- (mg L^{-1})	bw	0.03 ± 0.02	0.22 ± 0.27
	s	0.3 ± 0.2	0.7 ± 0.5
PO_4^{3-} (mg L^{-1})	bw	4.2 ± 2.3	4.5 ± 2.7
	s	5.3 ± 2.5	5.3 ± 2.4

At the two seasons of the year, a clear difference was observed between the bottom water salinity and the interstitial water one; the salinity was greater in interstitial water at north winds time and in bottom water at dry season. The oxygen concentration was also greater in the bottom water at the dry season; this agrees with the more electronegative values of Eh in the interstitial water. The pH was generally basic as much in bottom water as in interstitial water, with the exception of

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the bottom water that presented average values of neutral pH at dry season (Table 1).

The N (NH_4^+, NO_2^-, NO_3^-) concentrations presented contrasting values between the different sampled sites. The nitrogen concentration under ammonium form in north winds season in both biotopes, did not present significant differences. At dry season, the interstitial water ammonium concentration was most important than in bottom water and in the north winds season (Table 2). In north winds season, in El Sábalo site sample, the N-NO_2^- (0.079 mg L^{-1}) greater concentration in bottom water appeared, whereas in interstitial water the maximum value was in Punta Levisa (0.032 mg L^{-1}); at dry season there was a significant increase in interstitial water with values from 0.012 to 0.089 mg L^{-1} N-NO_2^- ; at this season of the year, in bottom water, the nitrites concentrations were very low (0.0002 to 0.0044 mg L^{-1} N-NO_2^-). Only the nitrate was relatively elevated in interstitial water at both seasons of the year (Table 2).

The values of materials suspended in bottom water were much higher at north winds time than in dry season (Table 2), because of the sediment resuspension at this season of the year; the highest values were encountered in the rivers mouths and the El Fraile estuary, and the lowest in the zone of the navigable channel.

At both seasons of the year the sandy-slit type of sediments predominated, although the greater proportion of slit and clays was encountered at dry season (Table 1), by the low sediment resuspension. The same behavior was observed in the values of organic matter percentage.

Microbial hydrolytic activity in bottom water.

The principal compounds analysis (PCA) results showed that there is a small relation between the DHA, the O_2 and salinity concentration in bottom water (Fig. 2). As it can be observed in tables 1 and 3, the greater values of DHA (65.5 ± 41 µg mL^{-1} INTF), oxygen (5.6 ± 0.9 mg L^{-1}), and salinity (20.8 ± 8.8 ppm) in bottom water were found at dry season, because at this season of the year the bottom water oxygenation was favored by

Table 2. Variation of the texture, organic matter concentration in the superficial sediments and suspended materials (SMA) in the bottom water in each sampling stations of and the two seasons of the year studied. nd= not determined

	Dry season					North winds				
Station	Sand (%)	Clay (%)	Silt (%)	OM (%)	SMA (mg L^{-1})	Sand (%)	Clay (%)	Silt (%)	OM (%)	SMA (mg L^{-1})
El Real	98	0.4	1.6	0.08	0.04	51	8	41	0.7	20.21
Chancarral	35.6	12.4	52	1.19	0.03	78	10	12	0.58	30.86
C. Norte	96	0.4	3.6	0.19	0.03	97	2	2	0.25	38.15
R. Basura	20	10.4	69.6	4.73	0.03	45	8	47	1.67	77.14
El Cocal	70	6.4	23.6	1.22	0.10	nd	nd	nd	nd	91
P. Levisa	70	6.4	23.6	3.19	0.02	87	4	9	0.3	NA
La Boya	70	10.4	19.6	1.47	0.13	75	10	15	0.94	167.9
El Fraile	32	14	54	2.19	0.05	43	7	51	2.17	82.91
La Palma	85.6	4.8	9.6	1.39	0.11	91	3	6	0.78	14.71
R. Sábalo	71.6	10.8	17.6	2.93	0.03	59	5	36	0.46	91.25

Receive: September 5th, 2013. Accepted: November 30th, 2013. Published: December 1st, 2013

Revista Digital del Departamento
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ISSN: 2007-5782

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the constant entering of coastal water through the mouth, favorable for the heterotrophic activity of the benthonic microbial community.

According to the correlation analysis (Fig. 2), in dry season, it was shown an inverse relation between the low AcPA concentrations and the low values of the SMA and temperature (Table 2). At time of north winds, the highest AcPA (2,1 ± 0,9 µg mL^{-1} p-nitrophenol) was obtained, since in this season the average water temperature was 30.7 ± 2.2 °C, that is adapted for an optimal catalytic action of the groups phosphate (Wood 1977). Nevertheless, a characteristic of the SMA in the water column is that they retain the group phosphate, avoiding that this element is available for micro plankton; due to the fact that the phosphorus is combine with organic molecules of high molecular weight and this prevent the catalysis of the reaction by the AcPA (López 2006).

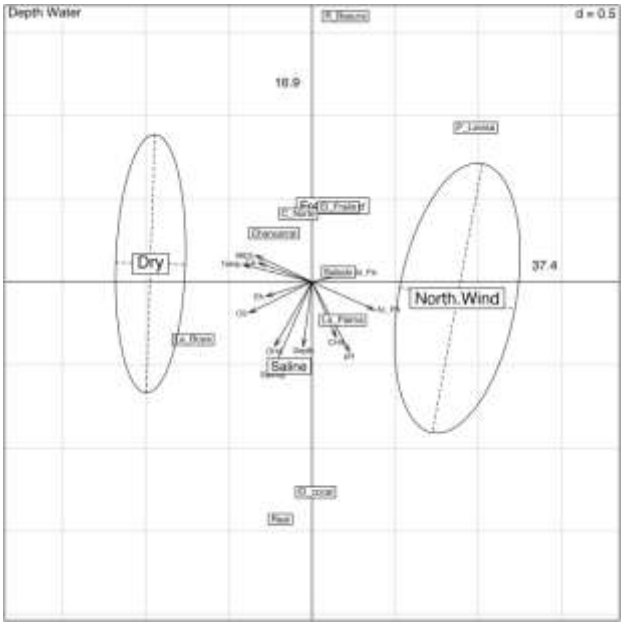

Fig. 2. Relation between microbial exoenzymatic activities and physico-chemical characteristics (vectors) of the environment according to a PCA statistical analysis in bottom water, on different sampling sites (squares), at time of north winds and dry season (ellipses). The numbers close to each axis are the percentage of variance for every eigenvectors. The scale of the graph is given by a grid which size is given in the upper right corner.

On the other hand, it was observed that Eh low values and low oxygen concentrations allow higher AlPA in bottom water at north winds season, mainly in El Sábalo and Punta Levisa sample sites (Fig. 2). Nevertheless, the values observed in AlPA at both seasons of the year were low because the pH average values were 7.9. It was reported that the maximum use of phosphate (HPO_4^{2-}) is carried out in a rank of pH between 6 and 7, since in an acid environment it precipitates as iron phosphate and aluminum phosphate quite insoluble, and in an alkaline pH, this precipitation takes form of also insoluble calcium phosphate compound (Wood 1977).

At north winds season, it was only observed the influence of the alkaline values of pH on the low CHA (0.3 ± 0.2 µg mL^{-1} N-acetylglucosamine), mainly in La Palma sample site; nevertheless, this activity could also be inhibited by the high salinity reported by El-Hamdaoui et al. (2008), who affirmed that the saline ions presence can be potentially toxic for the microorganisms, causing the diminution or inhibition of the enzymatic activity. This agrees with the low CHA found at dry season, when the bottom water salinity was higher than at north winds season.

Although the cellulolithic activity (AC) values were much smaller than the rest of the enzymatic activities, it was possible to observe that the highest concentrations were positively influenced by the highest temperatures in dry season, mainly in El Chancarral sample site; nevertheless, at north wind time, none influence of any physico-chemical variable was observed on AC. This can be explained by the fact that in the dry season, there is a greater concentration of organic residues constituted by cellulolithic compound deposited in the sediments. The low AC values found in this study can be due to the fact that the cellulose is a resistant, fibrous substrate, with a complex macro-molecule constitution (polioses and hemicelluloses), which are heteropolysaccharides compounds of high molecular weight, constituted by different monosaccharides (pentoses, hexosas and uric acids) units which causes that these polysaccharides are hard to hydrolyze, because only some bacteria and free life fungi present enzymes necessary to break links β-1,4-glucosidic. At this, it is to add that the

Receive: September 5th, 2013. Accepted: November 30th, 2013. Published: December 1st, 2013

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cellulose hydrolytic activity is optimal in neutral pH (6.5 to 7.5) medium (Deng and Tabatabai 1994; Gutiérrez *et al*., 2008), which is different from the alkaline conditions registered in this lagoon (table 2) (Fig. 2).

Hydrolytic activity in sediment

In the sediments, a significant increase in the concentrations of the DHA was observed (82.1±38.6 µg INTF), correlate with the highest concentrations of OM (1.9±1.4 %) in north winds time; this agrees with the published by Acosta and Paolini (2005) (Fig. 3), who affirmed that the type of OM or compounds incorporated in it is able to activate the native microbial biomass of the sediment; in addition, that the DHA is a reflect of the efficiency of the oxidizing activity of the sediment organic compounds. Nevertheless, this direct relation between the DHA and the OM was not observed with the same clarity at wind north time.

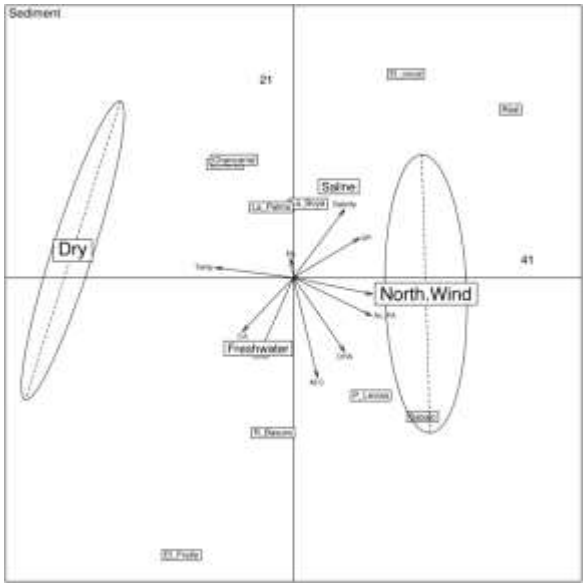

Fig. 3. Relation between microbial exoenzymatic activities and the physico-chemical characteristics (vectors) of the environment according to a PCA statistical analysis, in superficial sediments (1 cm depth), on the different sampling sites (squares), at time of north winds and dry season (ellipses). The numbers close to each axis are the percentage of variance for every eigenvectors. The scale of the graph is given by a grid, which size is given in the upper right corner.

The CA presented a slight increase in both seasons of the year, in comparison with the one found in bottom water; nevertheless, this increase was not statistically significant because the cellulolithic enzymes associate themselves quickly to the particulate material like clays, OM and humic substances, diminish or inhibit its activity (Gutiérrez et al. 2008).

On the other hand, the phosphorous activities (acid and alkaline) did not show significant changes in the sediment, staying almost in the same levels than in bottom water in at north winds time. At dry season, both activities presented very low concentrations in comparison with the ones found in bottom water. The PCA showed an inverse relation with the temperature unlike the ones obtained in bottom water (Fig. 3).

Like in the basic water the CHA was low; in spite of the great interest that exists on the activity chitinase in soils and sediments, the works carried out with this enzyme are very scarce and, due to this the scenarios and situations in which the CHA has been studied are very little, for this reason, the factors that effect to the evaluation method are not known thorough (Garcia et al. 2003).

Bacterial exoenzymatic activities.

Concerning the heterotrophic bacterioplankton and bacteriobenthos capacity to hydrolyze high molecular weight organic compounds, it was observed that in bottom water, during the dry season, there was a greater capacity to hydrolyse: > glucides (esculinase) > fatty acids > proteins > phosphor organic susbratum, this perhaps because at this season it exists a greater concentration of organic compound incorporated to the system by the pluvial contributions that constantly nourish the water column of these easily assimilable elements.

On the contrary, at the north winds time, the mainly hydrolyzed were> proteins >carbohydrates, > phosphorous organic compounds > carbohydrates >. In both seasons, the capacity to hydrolyse nucleic acids, the starch, chitin and the cellulose was not

Receive: September 5th, 2013. Accepted: November 30th, 2013. Published: December 1st, 2013

Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

Vol.2. No.4: 27-37. July to December. 2013.

demonstrated. On the other hand, at dry season, a good enzymatic expression of: >amylase, >lipase, > lecithinase and > phosphatase was observed in the sediment.

These results agree with recent investigations that have demonstrated that the organic compound decomposition is realized by a complex microbial association, mainly bacteria; at the beginning, the more available organic materials are used (mono- and disaccharides, amino acids, proteins), which is reflected in a high activity of extracellular enzymes like gelatinase, esculinase and phosphatase.

Later, the most complex compounds like the cellulose, lignin and chitin are hydrolyzed by other anaerobic microbial groups. Thus, the decomposition of these materials in the bottom water and the sediment constitutes a basic biological process in which the carbon is recirculated in the system.

Thus, we can say that the bacterial community in this ecosystem plays a fundamental role in the decomposition of the soluble and particulate organic matter present in the water column and the sediments, giving like result the recycling of inorganic nutrients like nitrogen, phosphorus, sulphur and other elements traces, which can mainly be used by the primary producers like the phytoplankton (Murrell 2003).

CONCLUSIONS

The variability registered of the microbial exoenzymatic activities is linked to the season of the year and the carbon content available in the Sontecomapan lagoon. The activity of dehydrogenase and phosphatases was the most representative in the ecosystem studied, whereas the cellulose and chitin, in spite of being the most abundant biopolymers, were the hardest substrates to hydrolyse since they are high molecular weight compounds which are generally mineralized by the anaerobic microbiota. The enzymatic activities

Table 3. Average and standard deviations of the microbial exoenzymatic activities *ex situ*. DHA (dehydrogenase activity); CA (cellulase activity); CHA (chitinase activity); AcPA and AlPA (acid and alkaline phosphatase activities).

Enzymatic activities ($\mu g\ ml^{-1}$)	Biotope	North winds	Dry season
DHA (INTF*)	bw	41.6 ± 36.6	65.5 ± 41.0
	s	82.1 ± 38.6	46.9 ± 17.3
CA (glucose)	bw	0.02 ± 0.01	0.1 ± 0.06
	s	0.14 ± 0.06	0.4 ± 0.44
CHA (N-acetylglucosamine)	bw	1 ± 2.5	0.3 ± 0.2
	s	0.3 ± 0.1	0.4 ± 0.4
AcPA (p-nitrophenol)	bw	2.1 ± 0.9	1 ± 0.4
	s	1.7 ± 0.5	0.002 ± 0.002
AlPA (p-nitrophenol)	bw	1.3 ± 0.5	0.9 ± 0.4
	s	1.3 ± 0.5	0.0009 ± 0.0005

*Iodonitrotetrazolium formazan

Receive: September 5th, 2013. Accepted: November 30th, 2013. Published: December 1st, 2013

values presented in this investigation were very low in comparison with agricultural soils studies.

ACKNOWLEDGEMENTS

This study was support by the Mobility ECOS-ANUIES-CONACYT Program (189448).

REFERENCES

Acosta Y and J Paolini. 2005. Actividad de la enzima deshidrogenasa en un suelo calciorthids enmendado con residuos orgánicos. Agronomía Tropical 55 (2): 217-232.

Álvarez S. 2005. La descomposición de materia orgánica en humedales: la importancia del componente microbiano. Ecosistemas 14 (2): 17-29.

Aminot A and M Chaussepied. 1983. Manuel des analices chimiques en milieu marin. CNEXO, BNDO/Documentation. Francia. p.396.

Ángeles-Vázquez JR. 2007. Tasas de fijación de nitrógeno atmosférico y caracterización química y molecular de bacterias diazotrofas microaerofílicas procedentes de sedimentos. Tesis de Maestría. Ciencias Agropecuarias. Universidad Autónoma Metropolitana-Xochimilco, México. 74 p.

Caldwell B. 2005. Enzyme activities as a component of soil diversity: a review. Pedobiología 49: 637-644.

Cowan ST and KF Steel. 1974. The principles of microbial classification. The philosophy of classification. Journal of General Microbiology 12:314-321

Curticapean M and M Dragan. 2007. The enzymatic activity from the sediment of the Gilau Dam Reservoir-Cluj Counti. Journal of Biochemical and Biophysical Methods 69 (3): 261-271.

Deng SP and MA Tabatabai. 1994. Cellulase activity of soils. Soil Biology and Biochemistry 26:1347-1354.

Dray S and AB Dufour. 2007. The ade4 package implementing the duality diagram for ecologist. Journal of Statistical Software 22:1-20.

El-Hamdaoui A, I Bonilla and L Bolaños. 2008. Salinidad: efectos del estrés salino sobre la fijación de nitrógeno en leguminosas. Universidad Autónoma de Madrid, España. p. 235.

Fernández L, M Sagardoy and M Gómez. 2008. Estudios de la fosfatasa ácida y alcalina en suelos de la región pampeana norte del área sojera Argentina. Ciencias del Suelos 26 (1): 35-40.

Frutos MD, J Blasco and A Gómez. 2004. Actividad fosfatásica en salinas de la Bahía de Cádiz. Ciencias Marinas 30 (3): 403-416.

García C, T Hernández, F Costa, B Ceccanti and G Masciandaro. 1993. The dehydrogenase activity in a soil as an ecological marker in process of perturbated system regeneration. p. 27-30. En: Proceedings of the Sixth International Symposium Environmental Biogeochemistry, Salamanca, Spain, 27-30 septiembre.

García A and JJ Ibañez. 1994. Seasonal fluctuations and crop influence on microbiota and enzyme activity in fully developed soils of central Spain. Arid Soil Research and Rehabilitation 8: 161-178.

García C, F Gil, T Hernández and C Trasar. 2003. Técnicas de análisis de parámetros bioquímicos en suelos: medida de actividades enzimáticas y biomasa microbiana. Mundi-Prensa. España. 215-237 p.

González FA, BA Vázquez, FS Villanueva and VG Ponce. 1994. Presencia de metales en sedimentos recientes y organismos de la laguna Sontecomapan, Veracruz, México. Hidrobiológica 4 (1-2): 35-43.

Gutiérrez V, A Pinzón, J Casas and M Martínez. 2008. Determinación de la actividad celulolítica del suelo proveniente de cultivos de *Stevia rebaudiana* Bertoni. Agronomía Colombiana 26 (3): 497-504.

Jeffries CD, DF Holtman and DG Guse. 1957. Rapid method for determining the activity of microorganisms on nucleic acids. Journal of Bacteriology 73:590-591.

Koroleff F. 1969. Direct determination of ammonia in naturals waters as indol-phenol blue. ICES, C.M. 1969/C: 9 Hydro Community.

Lányi B. 1987. Clasical and rapid identification methods for medically important bacteria. Prentice Hall Inc. California, USA. 137 p.

López R. 2006. Evaluación del efecto de molibdeno sobre algunos parámetros bioquímicos del suelo y la planta en Andisoles del sur de Chile. Tesis de Doctorado. Universidad de la Frontera de Chile. 115 p.

Morán-Villa V. 2007. Influencia de los parámetros físicos y químicos sobre las tasas de fijación de nitrógeno atmosférico y de desnitrificación en la laguna de Sontecompan, Veracruz. Tesis de Maestría en Ciencias Agropecuarias. Universidad Autónoma Metropolitana-Xochimilco. México. 75 p.

Murrell MC. 2003. Bacterioplankton dynamics in a subtropical estuary: evidence for substrate limitation. Aquatic Microbial Ecology 32: 239-250.

Receive: September 5th, 2013. Accepted: November 30th, 2013. Published: December 1st, 2013

Nelson N. 1994. A photometric adaptation of the Somogy method for determination of glucose. Journal Biological Chemistry 153: 375-380.

R Development Core Team. 2013. R: A language and environment for statistical computing. R foundation for statistical computing.Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Rodríguez R, G Godoy, G Morgan and RA Shelby. 1983. The determination of soil chitinase activity: conditions for assay and ecological studies. Plant and Soil 75: 95-106.

Strickland DHJ and TR Parsons. 1972. A practical Handbook of seawater analysis. Fisheries research Boar of Canada. Otawa. 321 p.

Tabatabai MA and JM Bremer. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology Biochemistry 1: 301-307.

Tramer J. 1952. Simple and rapid method for the estimation of bacterial phosphatases using di-sodium para-nitrophenylphoshate as substrate. Journal Dairy Research 19: 275-287.

Villegas M, N Aguilera and L Flores. 1978. Método simplificado de análisis para la clasificación granulométrica de los minerales del suelo. UNAM. Revista del Instituto de Geología 2 (2): 188-193.

Walkey A and IA Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic titration method. Soil Science 34: 29-38.

Winkler M. 1998. Tratamiento biológico de aguas desecho. México. Limusa-Noriega. 31 p.

Wood W. 1977. Bioquímica. Fondo Educativo Interamericano. México. 125-139 p.

ZoBell CE. 1941. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. Journal Marine Research 44:42-75.

Receive: September 5th, 2013. Accepted: November 30th, 2013. Published: December 1st, 2013