Use of Continuous Aeration Respirometry Method for the Prediction of Slightly Saline Waste Water Biodegradation

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Abstract

The microorganisms growth kinetics is determined by the physical and chemical characteristics of the environment in which they find themselves and multiply ; It is therefore essential to understand the effect of these environmental factors to master cell multiplication and hence the pollution biodegradation.

Respirometry has been used in recent years in the laboratory as an assessment technique of microbial activity and an effect detector of the contamination (presence of toxic, stress, increase or decrease in pH, temperature variation ...) on bacterial respiration and hence on the biological waste water treatment plant.

In this study respirometry has been used as a relatively quick and efficient means to detect the effect of the presence of a salt of up to 5 g / l (low salt stress) on the degradation of carbon and nitrogen pollution and on bacterial floc aggregation.

Keywords: Respirometry; waste water; salt stress; deflocculation

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1. Introduction

The salinity of a liquid is defined as the sum of cations and anions in it. The main salt present in almost inexhaustible quantities in the seas, oceans, salt lakes and also in salt deposits is sodium chloride (NaCl).

Salt is indeed a staple, used for food preservation as well as for the manufacture of chemicals products such as hydrochloric acid, sodium hydroxide (caustic soda), sodium bicarbonate, etc... . ; The industrial sectors of agro-food, chemicals / pharmaceuticals, leather, textile and petroleum are major consumers of

salt but also major generators of saline pollution (Lefebvre and Moletta, 2006). In addition, many coastal cities, such as Hong Kong use seawater for toilet flushing to save freshwater (Wu and al., 2008). All these wastewater sources are characterized by high salinity and a high organic and nutrient load.

The treatment of salt effluent is effected by physico-chemical process, but the biological pathway used for the treatment of organic matter in saline effluents could reduce the cost of the physical and chemical finishing. The biological treatment of such effluents can only be done by means of microorganisms tolerant to high salt concentrations (halophiles). These microorganisms are present in the far oceans but also in hypersaline environments such as salt marshes or alkaline lakes.

Moderate acclimation of activated sludge to high salinity is possible. Acclimation implies the exposure of non-salt-adapted micro-organisms to increasing salt concentrations in order to permit the obtention of satisfactory effluent treatment performance at a given salt concentration.The success of such adaptation depends on several factors, such as the type and growth phase of micro-organisms, as well as the rapid or gradual increase of salt concentration during acclimation.

The survival and diversity of microorganisms in hypersaline effluents and their ability to degrade organic pollution carbon, nitrogen and phosphorus is the subject of numerous studies (Tokuz,R and al ,1978;Woolard , C.R and al , 1994; Panswad,T. and al , 1999; Pernetti ,M.and al ,2005; ….).

The salt induced osmotic pressure which affects the metabolism of microorganisms. Indeed, the difference in solute concentration on both sides of the cytoplasmic membrane generates an osmotic pressure gradient. The free passage of water molecules through the cell membrane, allows then to reduce the concentration gradient. In addition to the passage of water molecules, bacteria can increase their internal solute concentration, through the production of potassium or sugar for example, the phenomenon of concentration which also regulates the osmotic pressure gradient.

Although some types of microorganisms (halophilic bacteria) need a salty environment to grow, the microorganisms most commonly used in biological treatment (non-halophilic bacteria) resent the salty environment.

respirometry is a more direct method for assessing sludge activity and thus toxicity to sludge. Many activated sludge respirometric methods are well-established and several standardized tests have existed for a long time (e.g., Organization for Economic Co-operation and Development (OECD), 1984; Environmental Protection Agency (EPA), 1996; International Organization for Standardization (ISO), 1986).

A continuous Aeration Respirometry is used in this study to predict whether or not to degrade the carbon and nitrogen pollution in the bacterial biomass of the treatment plant without adaptation in case of salt stress up to 5 g / l (low salt stress).

2. Materials and methods

2.1. Open respirometer experiments

Respiration was measured in an experimental set-up consisting of an open aeration batch reactor (500 ml working volume), as shown in Fig.1, the system was kept at a temperature of 20 ± 1 ° C using a thermal enclosure and mixing was obtained by magnetic stirring. Oxygen was measured with an oxygen electrode connected to OXI 357 oxygen measuring unit from WTW.

Oxygen measurements were logged by LINSEIS recorder, and respiration rates were then calculated by linear regression of all the obtained dissolved oxygen (DO) data.
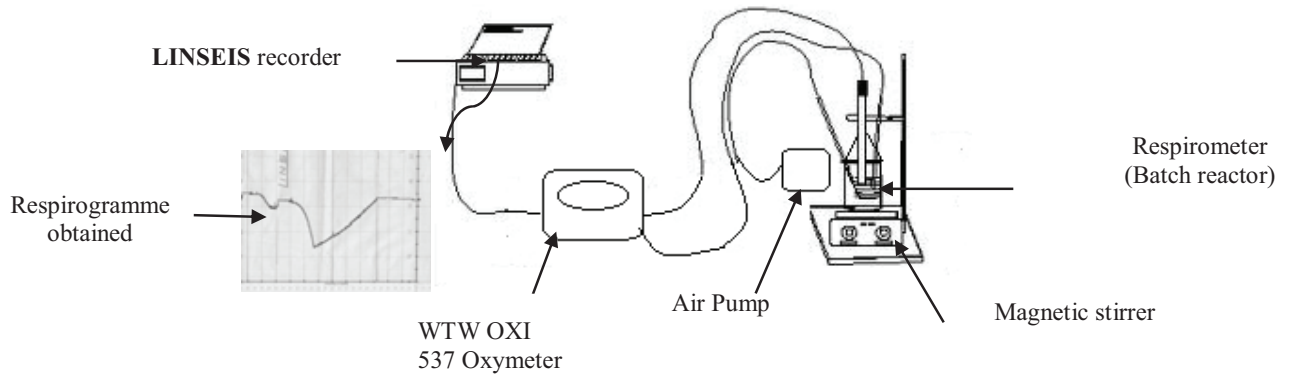
Fig.1. A schematic of the experimental open respirometer

2.2. Inoculations

There is a difference between halophilic bacteria and halotolerant bacteria. Indeed, the term "halophilic" means that microorganisms require the presence of salt (NaCl) in the medium for their growth. However, the term "halotolerant" means that microorganisms tolerate different salt concentrations but not required during growth (Table.1). (Kushner D. J., 1985)

To assess the influence of salt stress on the biodegradation of carbon and nitrogen composed and on the tolerance of microorganisms to adapt to this specific environment (the halotolerant bacteria), the respirometer were inoculated with fresh activated sludge obtained from a local sewage treatment plant **'IBN ZIAD'** Constantine, Algeria designed to treat 800l/s (6916 m^3/day) flow rate or 450000 population equivalent/d.

Sampling was carried out with a bottle of immersion (Fig .2); the bottle allows us to access multiple levels and allowing the sample to each desired level. The collected sludge was used immediately and no adjustment procedure was undertaken.

Fig.2. Sampling procedures in the « **IBN ZIAD** » station

Table.1 .Types of Halophilic Microorganisms

Categories	NaCl (M)		NaCl (g l⁻¹)	
	Range	Optimum	Range	Optimum
Halotolerant (no Halophilic)	0–1	0 <0,2	0–60	<10
Slightly Halophilic	0,2–2,0	0,2–0,5	10–115	10–30
Moderate Halophile	0,4–3,5	0,5–2,0	25–200	30–115
Extreme Halophilic "Borderline"	1,4–4,0	2,0–3,0	80–230	115–175
Extreme Halophilic	2,0–5,2	>3,0	115–300	>175

2.3. Synthetic saline wastewater

Synthetic wastewater used throughout the studies was composed of binary substrate consisting of sodium acetate and ammonium chloride and Salt (NaCl) its concentration was varied between 0 and 5 g/l. the injections of carbon and nitrogen to the reactor were such that the resulting chemical oxygen demand (COD) and $(NH_4^+ -N)$ in the reactor are equal to 15,2 mg/l and 1,6 mg/l respectively, hence the initial S_0/X_0 ratio is about 2,87mg COD/mg TSS for the carbon substrate and 0,30 mg $NH_4^+ - N$ /mg TSS for the nitrogen substrate.

Acetate and ammonia have been chosen because these substrates are major components of domestic waste water (Volskay and Grady, 1990) and give a well defined respirogram without the need for adaptation of the sludge (Z.Kong and al, 1995).

3. Experimental results and discussion

3.1. k_La and OUR_{end} assessment on the respirometer

The experimental procedure for the determination of endogenous respiration and the transfer coefficient of oxygen in the respirometer includes the following steps:

➢ The sample is left to the endogenous (in continuous aeration) since the day before the test so that all the substrate (carbon or nitrogen) is consumed by microorganisms, (Delgado L, 2009) recommends only 2 to 3 hours so that all residual exogenous substrate is consumed, after this step, the sludge reaches a stable respiration called endogenous respiration, This results in a plateau reached in terms of concentration.

It is necessary to know the saturation DO concentration in the mixed liquor medium, it is often estimated by the tabulated values for clean water, but can be measured by an oxygen sensor to saturation by aeration of the mixed liquor filtered (Yann Le Moullee, 2008), in this study the saturation concentration is determined for a sample decanted over 24 h (because the filtration of the mixed liquor is very difficult) the supernatant is recovered and then aerated to saturation at 20 ° C. Several experiments were conducted on the mixed liquor decanted the $S_{0, sat}$ is 8.98 mg O_2 / l at a temperature of 20 ° C, or this concentration is 9.2 mg O_2 / l for the clear water at the same temperature.

➢ The second step is the stopping of aeration and the third step to the resumption of supply of dissolved oxygen, the second and third step allows the determination of transfer oxygen coefficient k_La and endogenous respiration according to equation (1).

$$\frac{dS_0}{dt} = k_L a\left(S_{0,sat} - S_{end}\right) - OUR_{end} \tag{1}$$

Where S_0, $S_{0,sat}$ and S_{end} are the instantaneous, the saturation and the endogenous DO concentrations, respectively.

➢ The fourth step is the return to the level of endogenous activated sludge after resumption of aeration (**Fig.3.**)
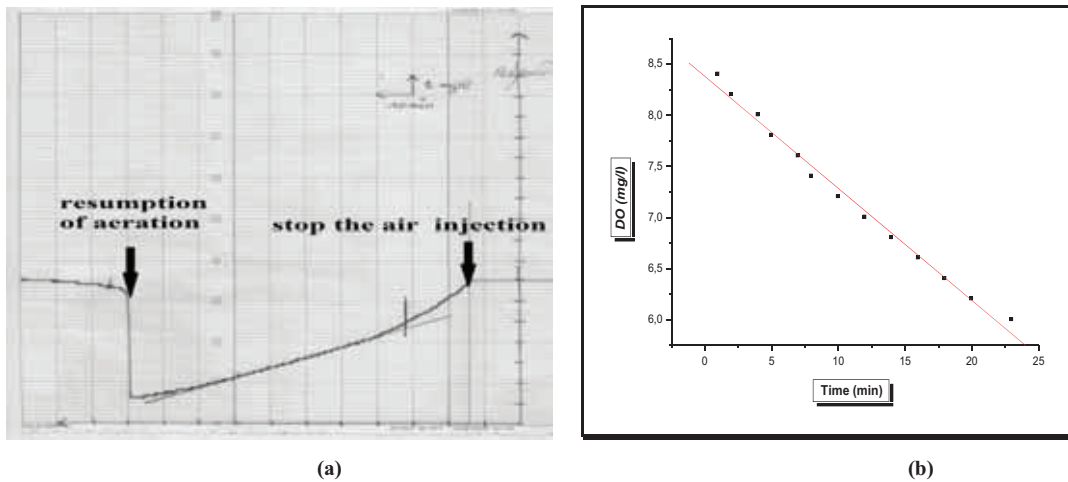

(a)

(b)

Fig.3. (a) Respirogramme obtained at 20°C, (b) Dissolved oxygen (DO) concentration as function of time in endogenous stage

Several tests were performed for the determination of $K_L a$ in the same operating conditions (agitation 100 r /min, T = 20 ° C), we obtain the following reproducibility:

Table.2. different values of $k_L a$ obtained in the different tests

tests	1	2	3	4	5
OUR$_{end}$ (mg/l.min)	0,0317	0,1892	0,1828	0,0497	0,0664
$K_L a$ (h^{-1})	1, 18	4	4,90	1,569	2,21

   The most important parameters to be adjusted in a respirometry test in continuous aeration is the aeration rate, agitation and reactor geometry, these parameters have been set in the various tests conducted to determine the transfer coefficient and endogenous respiration, but the reproducibility was not very good this can be caused by the following parameters:

- measurement of dissolved oxygen in the stop phase can be crucial distorted by the presence of some air bubbles around the electrode
- microbial activity cannot be identical in the same test

- although the liquid-gas surface area was minimized as much as possible during the experiments (in the stop phase) , It has been demonstrated that this transfer should not be ignored (A .Guisasola and al , 2005)

For this and according to the literature it is recommended to determine this parameter set for each experiment (Alexandre Baudouin, 2004).

3.2. Toxicity studies

To enable the operating staff of a treatment plant to respond quickly and effectively to an unusual situation, the presence of toxic waste water must be demonstrated as soon as possible. To this end, the observation of peaks respirometric parameters such as slopes of the peaks, especially the reduction of respiratory activity plays an important role.

So the approach to study the effect of the presence of low salinity on respiratory activity is as follows:

➢ After the step of determining the transfer coefficient and the endogenous respiration, the next step is the injection of a certain amount of binary synthetic substrate, sodium acetate and ammonium chloride, this then gives rise to an increase in oxygen consumption by microorganisms (autotrophic and heterotrophic) and thus a peak respirometric, once the substrate oxidized the dissolved oxygen concentration tends to return to the endogenous level as a result of the continuous aeration.

➢ This step consists in observing the effect of adding salt on the respirometric parameters of the peaks; this will be clear by the simultaneous injection of a quantity of salt varying between 1 and 5 g / l and the same amount of substrate.

Exogenous oxygen uptake (OUR$_{exo}$) data are central to respiration inhibition analysis; they can be obtained from equation (4), OUR$_{exo}$ curve reflects the kinetics of aerobic biodegradation of C and N substrates simultaneous by heterotrophic and autotrophic microorganisms
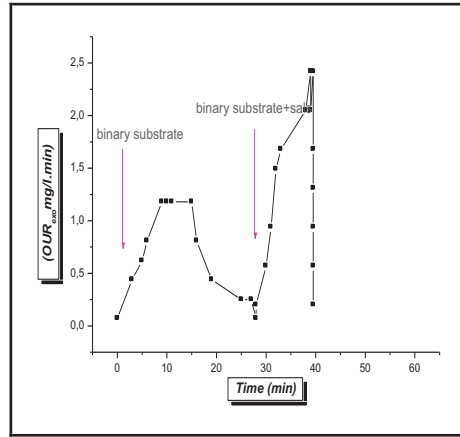
$$OUR_t = OUR_{exo} + OUR_{end} \qquad (2)$$

$$\frac{dS_0}{dt} = k_L a \left(S_{0.sat} - S_0 \right) - OUR_t \qquad (3)$$

$$OUR_{exo} = k_L a \left(S_{0.end} - S_0 \right) - \frac{dS_0}{dt} \qquad (4)$$

The evolution of dissolved oxygen concentration in experimental stages and the corresponding oxygen uptake rate (OUR$_{exo}$) as function of time with and without salt addition is represented in the following figures:
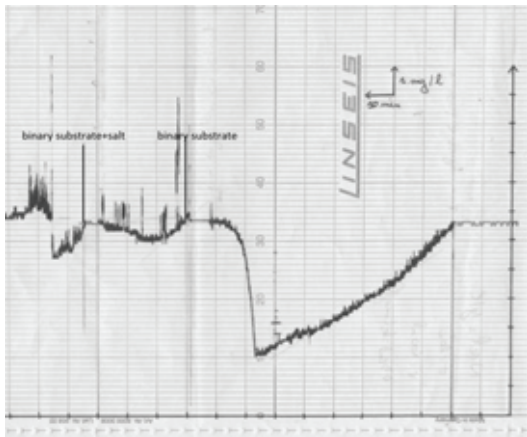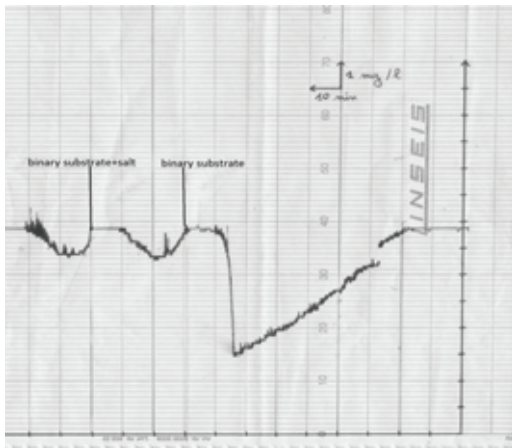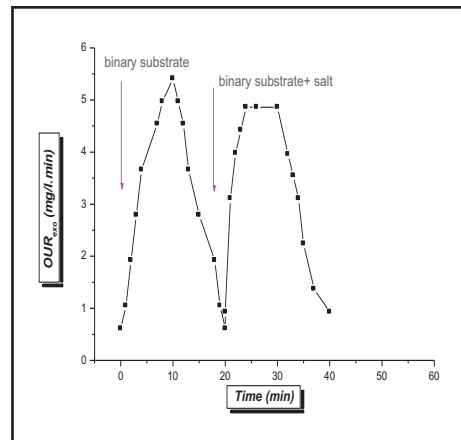
(a)   **(b)**

Fig.4. (a) Respirogramme obtained at 20°C without and with **1g/l** addition of salt, **(b)** the corresponding oxygen uptake rate (OUR_{exo}) as function of time

(a)   **(b)**

Fig.5. (a) Respirogramme obtained at 20°C without and with **3g/l** addition of salt, **(b)** the corresponding oxygen uptake rate (OUR_{exo}) as function of time
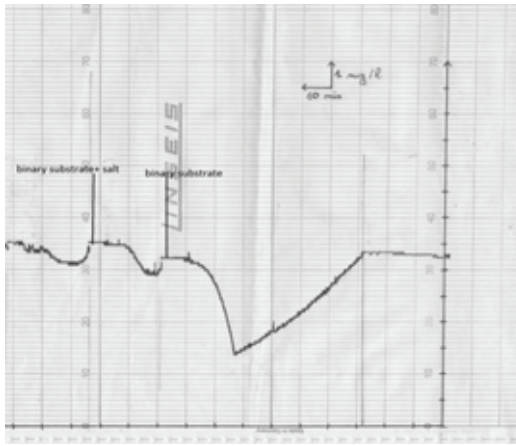
(a)   **(b)**

Fig.6. (a) Respirogramme obtained at 20°C without and with **4g/l** addition of salt, (b) the corresponding oxygen uptake rate (OUR_{exo}) as function of time
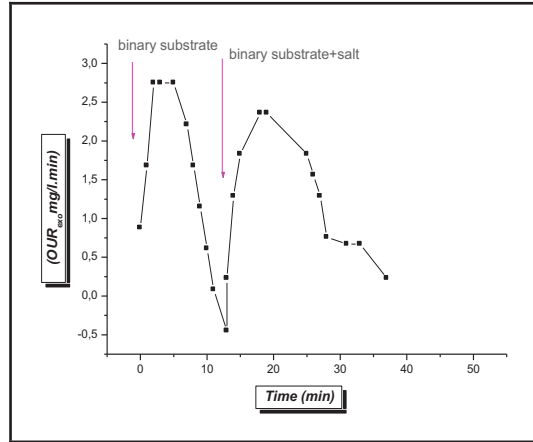

(a)   **(b)**

Fig.7. (a) Respirogramme obtained at 20°C without and with **5g/l** addition of salt, (b) the corresponding oxygen uptake rate (OUR_{oxo}) as function of time
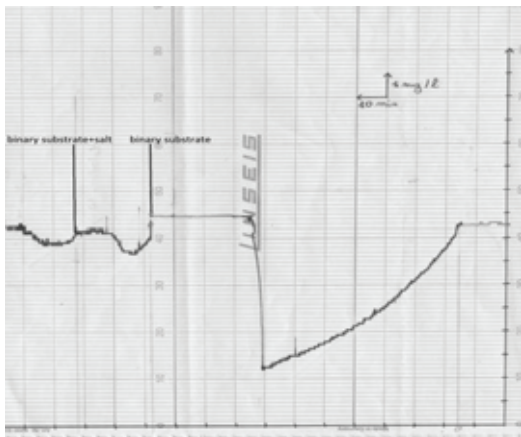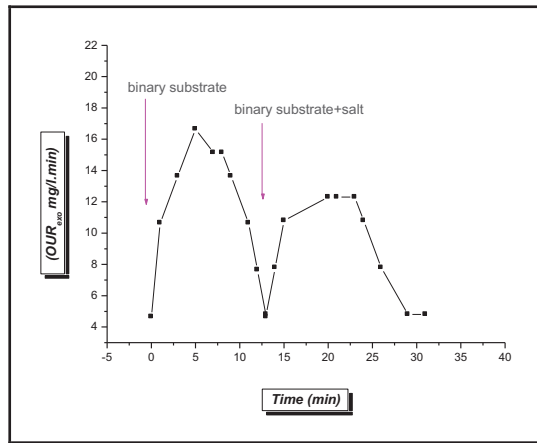
For concentrations up to 5 g / l of salt injected, inhibition is almost negligible; all respirogrammes obtained before and after injection of salt are almost identical (Fig.4, Fig.5, Fig.6, Fig.7).

3.3. *Effect of the salt addition on the amount of oxygen consumed by microorganisms*

The area formed under the curve for each peak (the corresponding oxygen uptake rate (OUR_{oxo}) as function of time), represents the quantity of oxygen consumed (necessary) for the assimilation of exogenous substrate added.

The reduction percentages in the amount of oxygen consumed with salt concentration added in comparison to the reference test is calculated according to the equation (5):

$$I\% = \frac{[\int OURexo(t)]_0 \, dt - \int [OURexo(t)]_I \, dt]}{\int [OURexo(t)]_0 \, dt,} \times 100 \qquad (5)$$

Where %I is the reduction of respiratory activity, expressed as a percentage, $\int[OURexo(t)]_0 \, dt$, the amount of oxygen consumed in the absence of salt (mgO_2/L) and $\int[OURexo(t)]_I \, dt$, the amount of oxygen consumed in the presence of salt (mgO_2/L).

Table.3. The amount of oxygen consumed and the percentages of reductions in the amount during the tests evaluating the effect of different pulses of salt on microbial activity.

Concentration of NaCl added (mg/l)	1	3	4	5
OUR_{end} (mg/l .min)	0.074	0.080	0.098	0.1
K_La (h^{-1})	1.85	4.36	2.67	15
the amount of oxygen consumed (mg/l) (without salt addition)	13.25	65.205	35	163.2
the amount of oxygen consumed (mg/l) (with salt addition)	14.36	69.205	33.23	168.9
I (%)	+8.37	+6. 13	-5.057	+3.49

Microorganisms commonly used to treat urban wastewater are poorly suited to the treatment of liquid wastes containing organic compounds and a high concentration of salts.

In response to high salt concentrations gradients bacterial cells will tend to empty their water by osmosis, and consequently to dry up. This phenomenon is called **plasmolysis,** causes a decrease in cellular activity. The presence of salt has an impact on the efficacy of biological treatment.

The salt concentration limit not to exceed in order to have a good biological degradation varies from one author to another subsequent work (Markez et al. ,1987) , have shown that media containing less than 1% salt, equivalent to 12 g .l^{-1} NaCl, are conducive to healthy development of non-halophilic bacteria. More recent studies (Woolard et al., 1995), made with traditional cultures of bacteria, used in domestic water treatment (activated sludge), show the difficulty of treatment of effluents containing 0.1 to 5% salt (1.2 to 60 g .l^{-1} NaCl). Kargi and Dincer (1997) observed that the effluent COD removal efficiency fell from 85% to 59% when salinity increased from 0 to 5%.

As respirogrammes obtained and the quantity of oxygen consumed before and after addition of salt it was confirmed that for low salt concentrations up to 5 g / l, the microorganisms was able to adapt by regulating the gradient osmotic pressure and therefore it was supported that environment for the degradation of the carbon and nitrogen substrate, no reduction in microbial activity (Table .3) was detected. On the other side a slight increase in microbial activity is detected due to microbial growth obtained in the first phase of the experiment (without salt addition) because in this study the substrate is much more abundant in solution than biomass (high ratio S/X).

3.4. Effect of addition of salt on the floc agglomeration

The main response to rapid changes in salinity is the release of cellular material, following the outbreak of the bacteria, resulting in an increase of soluble COD (Kincannon and Gaudy, 1968).

To confirm the results obtained from the respirometric tests, proving that no inhibition is detected for a salinity between 1 and 5 g / l, a microscopic examination is to establish a larger image (100 ×) using a microscope-type OPTECH assisted by a camera (Fig .8) no dispersion or deflocculation of floc was displayed and no outbreak of the bacterial cell was observed, some microorganisms are still mobile after the addition of 5 g / l of NaCl.
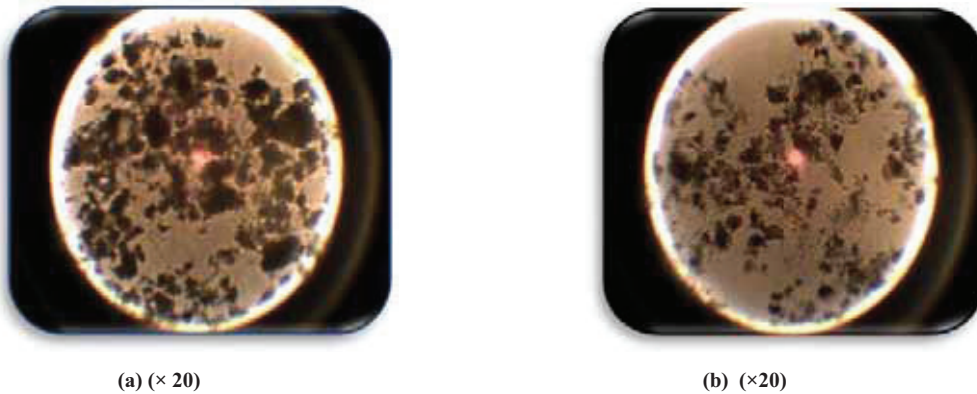

(a) (× 20)   (b) (×20)

Fig .8. Micrographs of sludge samples obtained ((a) without salt, (b) with addition 5g /l of NaCl

Conclusion

Given the variety of inhibitory compounds may enter accidentally in the treatment process of wastewater treatment plant, in an exhaustive list is almost impossible .They can be characterized by their purpose (respirometric technique) and not by their nature.

Respirometry seems to be a useful tool in monitoring and good start of treatment plants. Compared to many other methods, it is relatively easy to implement and low cost, the data could be used for simple characterization, control of the degradation process , to characterize more complex as the simulation and design of a treatment plant wastewater.

This study has shown that no reduction in microbial activity was detected at different NaCl concentrations: 1, 3, 4 and 5 g /l. using synthetic wastewater.

Because it difficult to compare removal efficiencies, and specific activities of microorganisms, this study was complete with a microscopic examination, no dispersion or deflocculation of floc was displayed and mobility of microorganisms are still good.

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