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THE EFFECT OF SALINITY ON BIOLOGICAL TREATMENT OF WASTEWATER FROM OIL INDUSTRY

(Master in Offshore Technology and Environmental Control)

Herimisa Andriamasinoro

Spring semester, 2009

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- Thanks for all support especially to my two lemons; my two sons “Dafnel and Baybol” who I dedicated this thesis.

ABSTRACT

High salinity may affect biological wastewater negative by reducing the growth rate of microorganisms. Wastewater from oil industry often contains high salinity which could be problematic to treat. This thesis is to evaluate the effect of salinity on biological treatment by performing laboratory test with variable salinities. In addition, microbiological investigation performed with microscopy and growth culture.

Keys words: salinity, bacteria, wastewater, oil industry, substrate removal

LIST OF FIGURES:

LIST OF TABLES:

Contents

INTRODUCTION

With recent technologies advances, oil and natural gas ensure fairness in the marketplace for business and supply the global energy. Moreover, Petroleum production, refining and transportation became more efficient and mature.

By implication, many units generate wastewater stream which has significant sources including water produced with crude oil, rain water and wash down water (Arnold et al., 1999). Those streams contain high concentration of salt and total dissolved solids.

Aware to possible contamination and regulation, the water cannot be disposed on the surface in offshore location and must be injected to an acceptable disposal formation. Zero harmful discharges (ZHD) to the North Sea from the Norwegian continental shelf (NCS) have been the environmental goal established by Norwegian authorities in agreement with the operators on the NCS (Eilen, 2007). In order to find an effective way of wastewater treatment, biological treatment using activated sludge is the most common (Ydstebø, 2009), efficiency and true destruction of organic compounds. It should be noted that new discharge permits require high removal efficiency not only for organic carbon but also for solids and most recently for nitrogen.

The purpose of wastewater treatment is to remove pollutants that can harm the aquatic environment if they are discharged into it. In order to accomplish this challenge, some microorganisms is used to convert the organic compounds using them as a source of energy and food for its growth result in generation of biomass, CO_2, water….. This information is scattered among thousands of books such as (Degremont, 2005), (Satin and Belmir, 1999)….The treatment is in two phases: contact with the biomass and wastewater in a reactor and separation of solids from the liquid purified by decantation. Its excellent performance of

remediation is with efficiency greater than 95% removed superior to other processes (Blassompiere, 2007). It should be noted that each source produce wastewater with specific characteristics. In this section, the type of effluent to be treated “salty” may be inducing a difficulty to control the treatment. For this instance, a study of effect of salinity on biological wastewater treatment from oil industry is conducted.

Salinity has generally a dual effect on growth rate based on Oxygen Utilization Rate (OUR), Volatile Suspended Solids (VSS) and substrate removal via osmotic effect on microorganisms and specific ion toxicities. Bacteria which take up salts have a higher tolerance and greater ability to break down and/or store high concentration in internal body without affecting cell process. Such case gives good result of degradability of organic compounds by measuring Total Organic Carbon (TOC) in wastewater. But it should not negligent that salt can be precipitate with water. This phenomenon can contribute to the quality of effluent.

After a brief overview of biological treatment, the first chapter provides a summary of technology used by SAR treatment. The second chapter focuses on various range of methods used to control and analyze the effect. While the last chapter illustrate the results obtained following by discussion of the specific impacts associated with them.

I. BACKGROUND AND LITTERATURE

I.1. OVERVIEW

I.1.1. Oil industry wastewater characterizations

The Exploration and Production (E&P), oil and gas industry generate a significant wastewater volume from the production and processing of oil and gas products. The characteristic of those wastewaters are dependent upon the method of recovery and the nature of the formation (Elsevier, 2001) in addition to methods of downstream processing (refining). It is apparent that this type of wastewater could vary depending on the producer, area of production and history of the exploration. The literature contains numerous articles concerned the description of this wastewater, of which some reelected papers are referred in this thesis:

Carmickael and Stezepek, (1987) explain that the basic uses of water in Chemical Petroleum Industry (CPI) are cooling water, steam generation and process water that account approximately for 80%, 5% and 15% respectively of intake water. In addition, (E-Razo et al., ?) underline that expect of wastewater from production, the CPI provides also storm water runoff and sanitary wastewater. Produced water can occupy a significant amount of the discharge and (Anonym , 2007) reveal that it contains a mixture of dissolved and dispersed oil, chemicals and fine solids from the reservoir. In 2002 on the NCS, produced water represented 120 million m^3 and is expected to increase to 180 million $m^3$by 2008 (Anonym, 2005). Keenan (1997) indicates that the wastewater may contain oil hydrocarbon carried from incomplete emulsion, certain level of hydrogen sulfide (H_2S), *Ammonia* and *Phenolic acids*. A Relatively, high level of suspended solids and dissolved solids are usually observed. The increasing environmental awareness led the Norwegian Petroleum Directorate (NPD), Norwegian Pollution Authority (SFT) and Oil Industry Association (OLF) to develop the zero harmful discharge strategy. This consists on enforcing the standards for maximal admissible concentration of discharge which results in more and more strict permits.

Produced water is important to remember that those streams can induce an adverse effect to certain habitat or vital resources of marine environment if it is not treated before discharge.

Awareness of the opportunities and the future of exploration were emphasized by Veggeland in (,): “Half of oil and gas in Norway estimate to be sold in 2020 has not yet been discovered (NPD, 2009). A Substantial number of these deposits are probably located in the Northern area”. This will tend to affirm that the wastewater production will continue to rise with oil production. The treatment of those streams will be an environmental challenge and enforce a competitive approach face of internationalization for the process industry.

I.1.2. Mechanism of biological treatment”Activated Sludge”

Compared to the other environmental problems, wastewater management have for long time received large attention. Also, many companies, interests and operators of oil and gas orient towards the preventive and corrective action for environmental protection. To satisfy the environmental requirements, development and use of wastewater treatment should be a priority. Biological treatment is one method by using Activated sludge (Ydstebø, 2006). The principle is to make contact of the wastewater and the microorganisms population. The soluble solid and organic matter is removed from solution by biological metabolism. Oxygen is consumed by the bacteria and new cell mass is synthesized. The treatment efficiency is determined by the time required:

- Hydraulic Retention time (HRT): the time by which the biomass degrade the COD in wastewater (Liquid retention in reactor).
- Sludge Retention Time (SRT): it is a time of which the sludge remains in the reactor.

It should be mentioned that bacteria is the main actress of metabolism in activated sludge and the other life forms present in activated sludge such as protozoa, metazoans,… are essential

for the proper balance of the ecosystem. Those species vary depending on the type of treatment plant and the conditions of the treatment operation (Pryd et al., 1990). Most frequent bacterial genera are *Acinetobacter, Citromonas, Chromobacterium, Flavobacterium, Flexibacter, Micrococcus, Pseudomonas and Zooglea* (Eckenfelder and Grau, 1992). Bacteria of the other genera can be abundant under specific conditions such as extreme temperature, pH or substrate composition...

I.1.3. Microbial perspective

The prokaryotes are microscopic organisms' single cells. In order to accomplish the biochemical reactions for growth, bacteria need energy permanently. Ydstebø, (2009) describes in details the bioenergetics involved for microbial process (Figure 1)

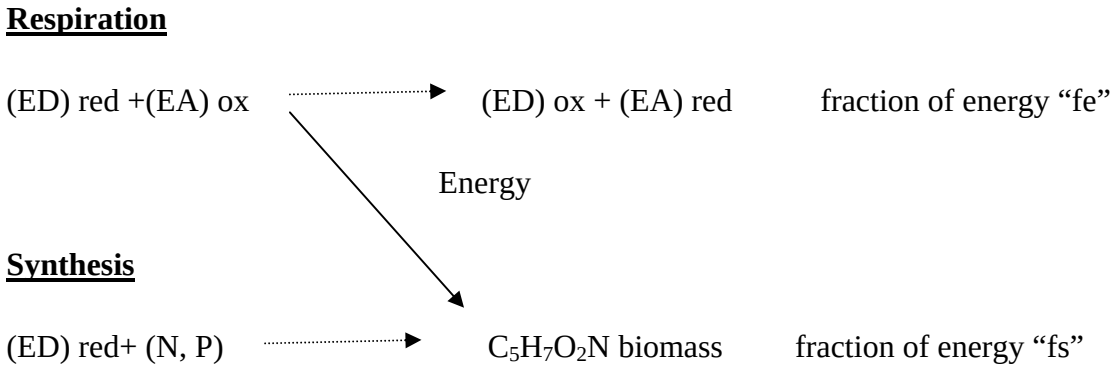

Figure 1: Bioenergetics of microbial processes

The energy is gained from substrate (electron donor "ED") which is present in wastewater and located outside the cell membrane. Depending on the species of bacteria, the requirement and the preferred compounds of how substrate and uptake may vary. In general, all bacteria need water, energy source, carbon source, nutrients and others mineral compounds. Those elements must be presented in sufficient amounts for the growth and maintenance of bacteria. A part from those nutritive elements, the presence of growth factor is necessary and important to satisfy the requirement and maintain the life of microorganisms. (Wesley and Grau, 1992) define the compounds available in wastewater as exogenous substrate. Also

(Ydstebø, 2006) named the substrate as electron donor and oxygen electron acceptor in aerobic processes.

The compounds in wastewater will be present as small or large molecules or as suspended solids depending on the nature of the wastewater. Clearly, the small molecules will be rapid taken up and utilized by bacteria and is called "Ready biodegradable Organic Compounds" (RBCOD) as they do not need any transformation before being used by the bacteria (Ydstebø, 2005). Large molecules with high molecular weight, colloidal and suspended organic matter must be hydrolyzed to simple molecules before utilization and is called "Slowly Biodegradable Organic Compounds" (SBCOD). This last type must be converted and restructured into small molecules before utilization. Bacteria secrete enzymes that break down the compounds into soluble forms so they can pass through the cell wall and into the cytoplasm (Figure 2). This phenomenon is conducted with an exothermic reaction that produces energy (Bassompiere , 2007). The derivate compound can be synthesized for growth or stored for a long period of time without expenses. The latter process is called "substrate Storage" and is frequently observed in biological wastewater treatment.

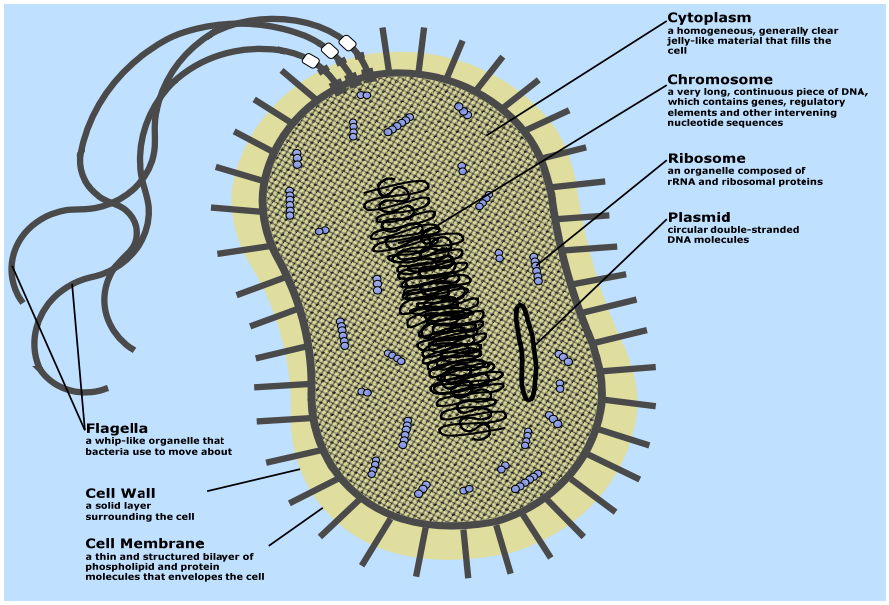

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Figure 2: Morphology of bacteria

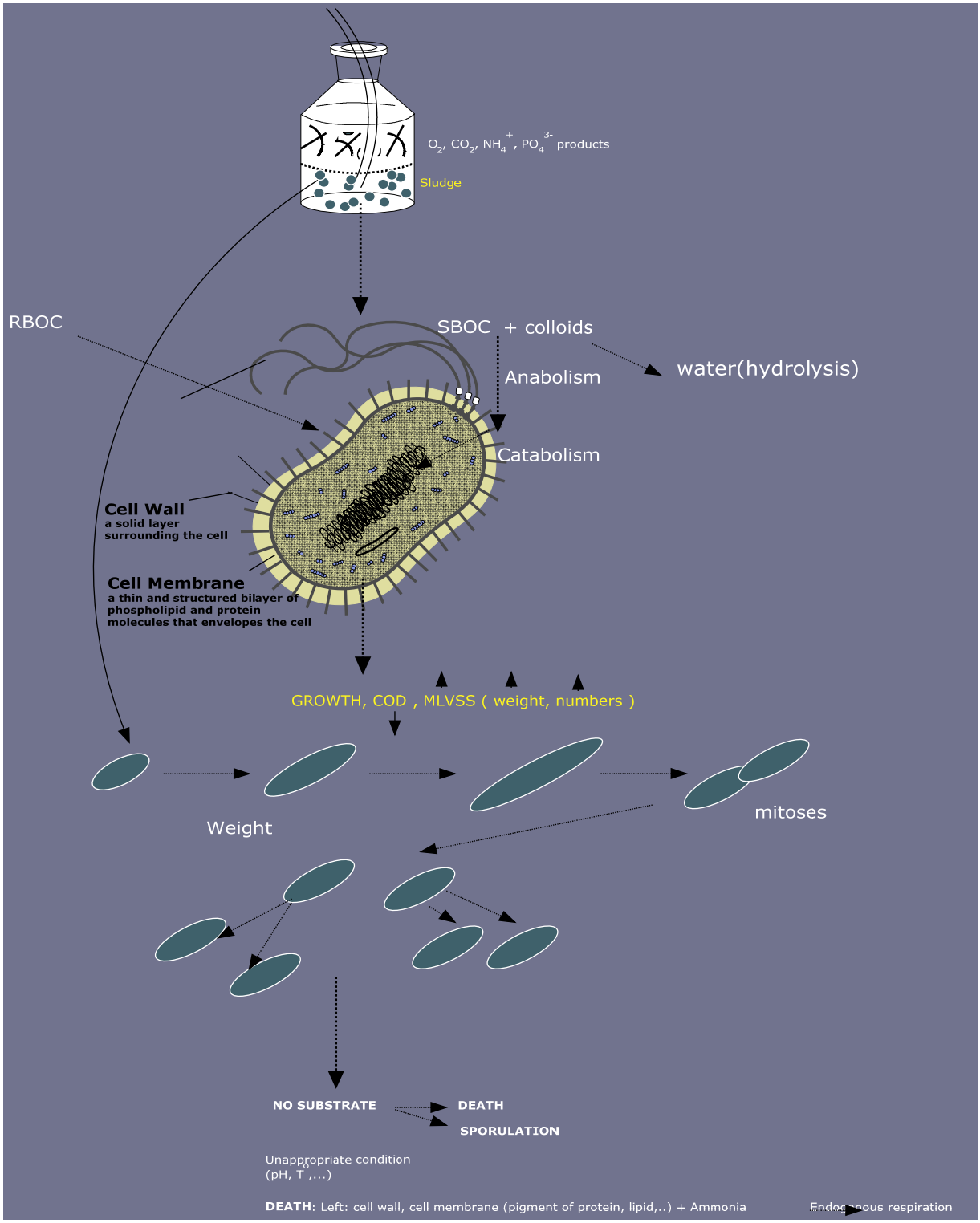

O_2, CO_2, NH_4^+, PO_4^{3-} products
Sludge
RBOC
SBOC + colloids
Anabolism
water(hydrolysis)
Catabolism
Cell Wall
a solid layer surrounding the cell
Cell Membrane
a thin and structured bilayer of phospholipid and protein molecules that envelopes the cell
GROWTH, COD , MLVSS (weight, numbers)
Weight
mitoses
NO SUBSTRATE
DEATH
SPORULATION
Unappropriate condition
(pH, T°,...)
DEATH: Left: cell wall, cell membrane (pigment of protein, lipid,..) + Ammonia
Endogenous respiration

Figure 3: Process of degradation

The colloids (0.08-100 um) present in wastewater are insoluble for the bacteria. Through hydrolysis, a water molecule is attached to one fragment of the parent molecule such as a polymer. This will allow the compounds to become more soluble, diffuse to the cell membrane and transported into to the cells internal body. The whole of those phenomenon cited above is called “Anabolism” corresponding to synthesis of new cells in growth.

When bacteria synthesize new cells they require energy. This phenomenon is called “catabolism”. The synthesis is following of endothermic activities which absorb energy from the surroundings. This reaction leads an increasing of ATP which is followed by increasing weight and volume of the single bacteria. At a certain volume, the bacteria then stop to grow and start to reproduce. The reproduction can be sexual or asexual reproduction and called “binary fission”. The single circular chromosome duplicates and results in 2 chromosomes. The cell elongates and separates into strands. The cell membrane grows inwards and the cell wall forms two separate daughter cells each with a chromosome. Finally, bacteria increase in number and all of the substrate are taken up. This induces bacteria in an inappropriate and critical condition which mislead to the death of some cell or transformed to spores. The cell membrane of the death microorganisms accumulates in the reactor. Adding sufficient nutrient, the cell compounds from the dead cell can be used again as food for the growth of the remaining microorganisms. (Ydstebø, 2009) named this phenomenon as endogenous respiration. It should be precised that the cell membrane contains protein and lipid.

I.1.4. Environmental factors affecting biological treatment

The environmental factors influence the growth of biomass it cannot act independently. In addition, the modification of one parameter can affect the modification of the others. Under these conditions, there main environmental parameters were:

Temperature: Higher temperature increases the growth rate of bacteria. Bacteria are normally divided according to temperature optimum:

- Thermophiles : optimum temperature(~55 deg C)
- Mesophlies : optimum temperature (~37deg C)
- Psychrophiles : optimum temperature(~10 deg C)

pH: Microorganisms have a range of optimum pH for carrying out its vital function in the range 6 to 8. Those bacteria are classified as neutrophiles such as *Escherchia coli.* Other bacteria prefer to grow at alcalin pH (>8) like *Pseudomonas sp*. Finally, the optimum growth conditions of acidophiles is <6 such as *Lactobacillus sp.*

Oxygen: Bacteria have a various mode of respiration; some needs oxygen for their growth and others not. It distinguished:

- Bacteria strictly aerobic: oxygen is necessary for their multiplication. (e,g *Pseudomonas sp.)*

- Facultative bacteria which growth is not affected by presence or not of the oxygen (e.g. *Escherchia coli*) .Oxidation and fermentative metabolism may also occur.

- Bacteria strictly anaerobic: it is developed only under the absence of oxygen (e.g. *Clostridium sp*.)

Salinity: In general, the bacteria have a good tolerance of salt. Certain bacteria halophiles need Sodium chloride (Nacl) for their growth.

I.2. SAR TECHNOLOGY

I.2.1. Design approach of SAR Technology

SART is a full service wastewater engineering company specializing in treatment of wastewater from oil industry. It gives turnkey facilities on operational support. To provide the highest quality and best value service to the clients, the company has developed a multi- stage process for treating wastewater from the oil industry (Figure 4).

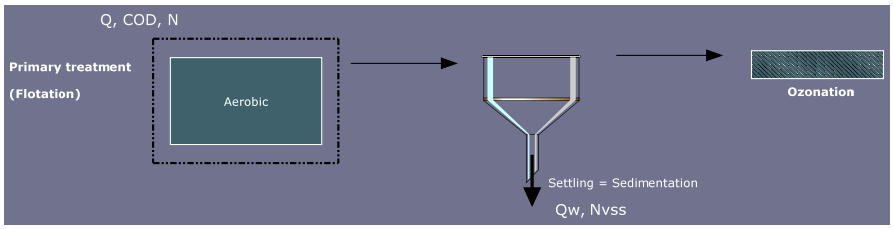


Figure 4: Process of treatment

I.2.2. Objectives

The goal is the establishment of new technology for solving and treating wastewater which enhance the efficiency of existing requirement and reduce discharge and its contribution to the global community. In this case, the effect of salinity on biological degradation of organic compounds in wastewater from oil industry.

II. MATERIALS AND METHODS

II.1. Experimental setup

The experiment on investigating the effect of salinity on biological treatment was done with two digestors of 2l (Figure 5). This consists of doing a comparative study of how efficient is the removal of compounds content in wastewater at different salt concentration. The period of experiment started at 09 February until 29 March.

Figure 5: Digestors materials for the experimentation

The experiment was performed with different conditions such as:

- 3.4 % of salt
- 6,9- 12% of salt
- Salt shock conditions

The room temperature was about 25 degC +/-2. The air pressure compressed to 1 bar is equipped. The air which flow to each digestor is humidified by bubbling in water and then transferred to the digestor.

II.2. Experimental procedure

The biological sludge and wastewater was collected from the activated sludge tank of SAR Technology treatment plant. The collected sludge is aerobic activated sludge and the wastewater was treated chemically for oil and suspended removal. For each digestor, 150 ml of sludge was introduced following by feeding wastewater and nutrients until 1000ml. The characteristics of the nutrient solutions (Macronutrients, Micronutrients) are shown in the appendix 1. The mode of feeding was every in each 2 days and the sludge remained in the digestor until the final experiment (50 days). From the beginning of cycle until the end, the temperature, pH, conductivity salinity and oxygen utilization rate (OUR) were measured. The total suspended solids and volatiles suspended solids were the parameters used most commonly analyzed to follow the biomass growth. Other complementary analyzes are also conducted in the laboratory for providing information and measurement of growth. This can be done by Total Organic carbon (TOC) analysis. The importance of this analysis is also a characterization of the “real” wastewater and effluent. TOC is an indirect measurement of organic matter as the carbon content in such compound are about 50% by weight. During the experiment, TOC analysis was tested at the end of the cycle. Also, the decanted effluent at the end of the cycle was analyzed for the concentration of phosphorus and nitrate. A microbiological examination was performed for getting information that enables to enumerate the biomass population. The data was evaluated in order to determine the characteristics under different conditions.

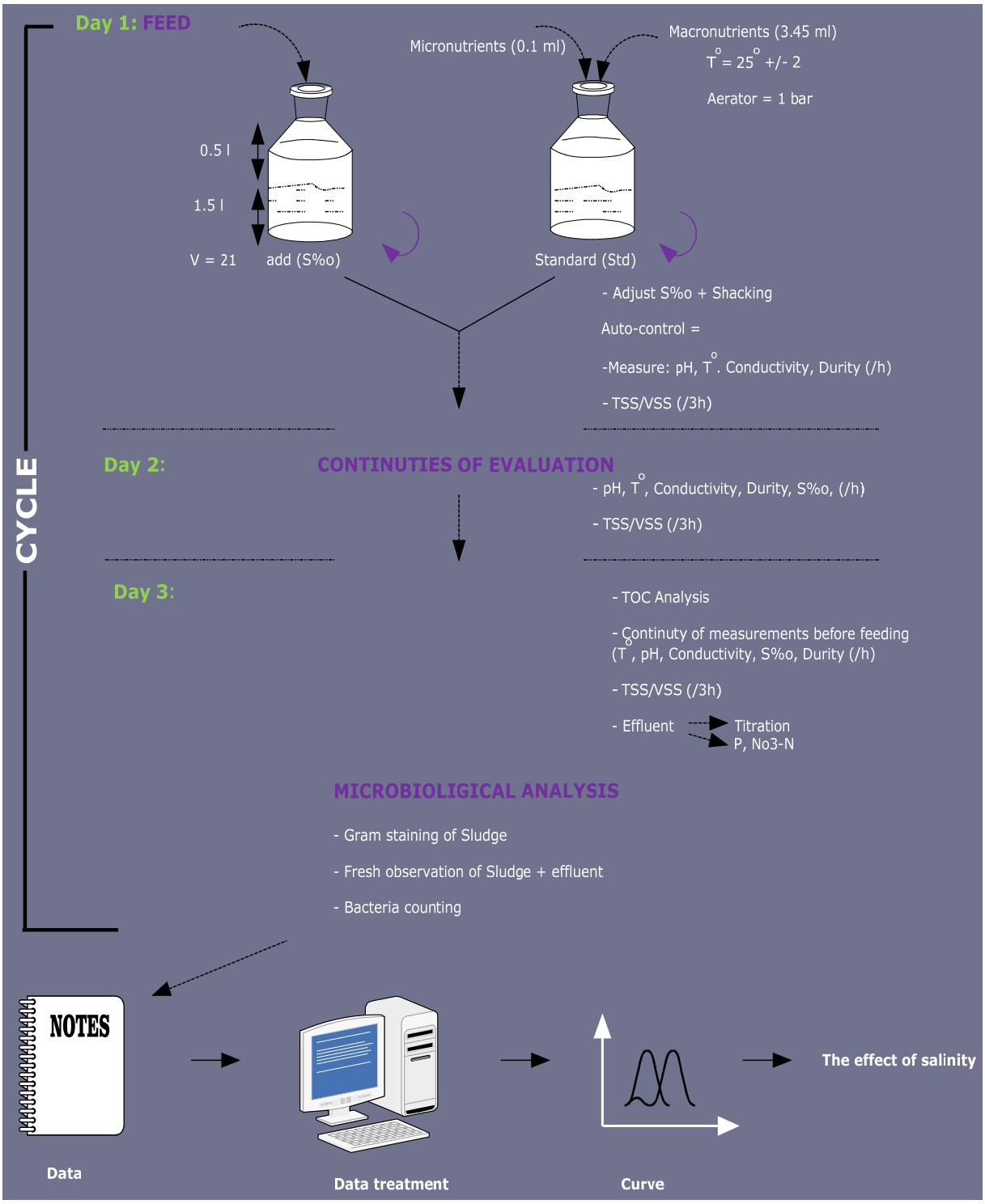


Figure 6: Process of evaluation

II.3. Analytical techniques

II.3.1. Determination of experimental conditions

Temperature, pH, Oxygen utilization rate (OUR), conductivity and salinity were determined with:

- Oxymeter : reference cell ox 325(Figure 7)

Measure: 0-50 mg.l -1 02(resolution 0.1 mg/l)

- Conductivity : reference Multi340i (10 uscm -1 to 500 ms /m-1)

Resolution 1 uscm-1(Figure 8)

Figure 7: Oxymeter

Figure 8: Conductivity

II.3.2. Determination of Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

- Using GF/C glass –fiber filters with 1 um pore size.
- Dry the filter at 105 deg C for at least 15 minutes prior to weighting to ensure they are completely dry.
- Weigh on a balance with 4 decimals (0.0000 g) (Figure 9).
- Measure an appropriate sample volume to be filtered in a graduated cylinder. The volume depends on the amount of solids present in the sample.

- Samples with high solids content should be allowed to settle (30-60 minutes) prior to filtration. The clarified liquid is filtered first and the concentrated sludge is added at the end. In this way, the filtering procedure goes much faster and the result becomes more correct. A small amount of distilled water is used to flush out any solids left in the cylinder.

- If any analysis is to be done on the filtered sample, then the volume of added distilled water must be noted, in order to calculate the dilution of the filtrate.

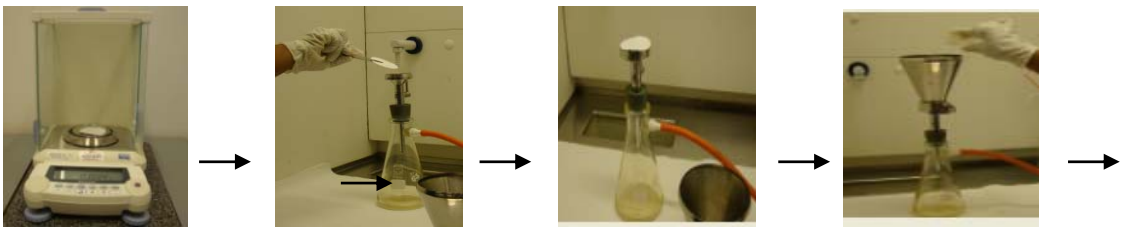
- NB: If the filtrate is to be used for analysis, detach the vacuum pipe before turning off the water, otherwise water from the tap is sucked into the container with sample.

- Volume to be filtered(approximates):- Low TSS(<50 mg/l): 100-300 ml (turbidity<50)- Medium TSS (50-300 mg/l) : 50-100 ml (Turbidity 100-200)- High TSS : (>300 mg/l): 10-25 ml (Turbidity >200)

- After filtration, dry paper (+TSS) at 105 deg C for at least 2 hours. Take paper out and weigh immediately. If the paper is exposed to air for longer period, moisture in the air may change its weight. Alternatively a closed moisture-free container can be used for transporting the dry papers to the weights.

- For determination of VSS, the papers are combusted in an oven at 550deg C for an appropriate period (usually 30 minutes). During this period, all organic compounds are burned off and the remaining solids on the filter paper are inorganic suspended solids (ISS).

- Weighing the paper will provide the fractions of the initial solids that were organic and inorganic, respectively.



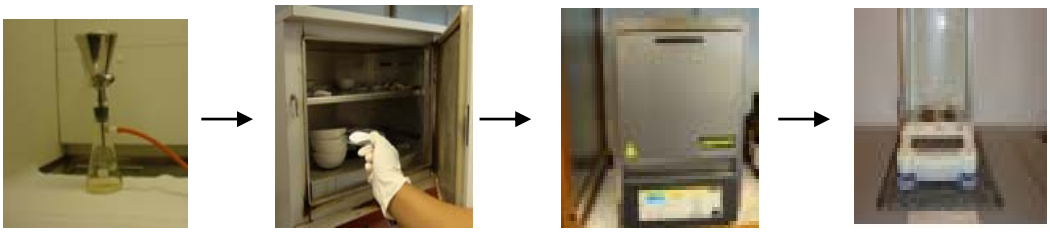


Figure 9: Filtration process

II.3.3. Analyse of Orthophosphate (P) and Nitrate (NO3-N)

The reactive P and N03-N are directly measured on filtered samples on the ion chromatograph (Dionex ICS- 3000) (Figure 10). After filtering for TSS/VSS determination, must be the filtrate b filtered through a 0.2 um syringe filter for more particle removal since the tubing in the IC is very small. Standard solution is made by K2HP04 to 100 mg/l P and calibration of the instrument is done within an appropriate range (depending on concentrations in samples). Previous calibrations can be used and tested by analyzing standards.

Figure 10: Dionex ICS- 3000

II.3.4. Total organic carbon (TOC)

A Schimadzu Total organic carbon analyzer model TOC 5000A (Figure 11) is used for determination of TOC. Total Carbon (TC) is determined by injecting the sample to a column with a platinum catalyser that is heated to 680 deg C and combust all carbon to C02- gas that

is transferred to a reaction vessel. The sample is then cooled and dehumidified and passes a halogen scrubber before it enters the chamber where C02 is detected with a non- dispersive infrared gas analyzer. The gas analyser produce an analogue signal corresponding to a curve on the display with the area under the curve corresponds to the mass of carbon in the sample. In order to determine TOC, inorganic carbon (IC) in the sample also must be determined. And TOC will appear as the difference between TC and IC.

Figure 11: Schimadzu TOC 5000A

II.3.5. Biological analysis

i. Microscopic observation" fresh state"

Many methods exist for qualitative observation of microorganisms. Microscopic observation is the simplest means of establishing a general picture of the microbial community on a sample. It requires on placing a known mass of sludge and observes directly to microscopy. On using Leica program (Figure 12), the population on the sample can be taken a photographed.

Figure 12: Microscopy related to Leica program

ii. Gram staining/

- Add crystal violet stain over the fixed culture (Figure 13). Let it stand for 10 to 60 seconds; for thinly prepared slides and wash off the stain. Add the iodine solution on the smear, enough to cover the fixed culture. Let stand for 10 to 60 seconds.

- Add a few drops of 95% alcohol for 30 seconds so the solution trickles down the slide. Rinse it off with water after 5 seconds. Counter stain with 0.25% safarin for 40 to 60 seconds. Wash off the solution with water.

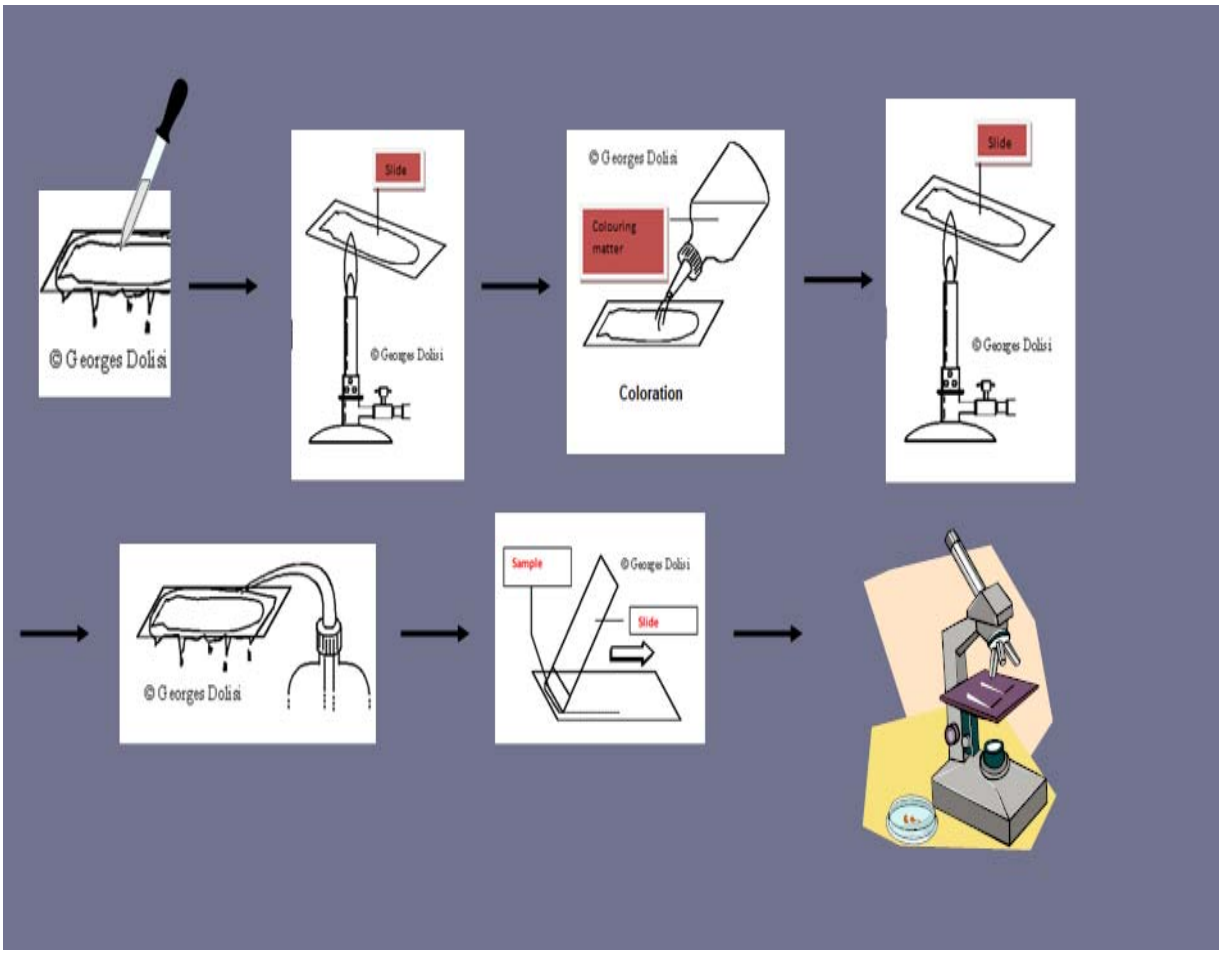


Figure 13: Gram staining

ii. Bacteria accounting

The sample to be counted must be dilute. Several 10 fold dilutions of the sample are commonly used. For accomplishing this dilution, 10 ml dilution need 0.1 ml of sample with 9.9 ml diluents (distilled water). And serial dilution are needed to reach the final dilution desired (10^{-9}) (Figure 14).

For preparing the medium, some chemical products are mixed for achieving the right nutrients solution for bacteria growth. This includes:

- Protease peptone (20 g)

- Beef extract (3 g)
- Yeast extract (3 g)
- Malt extract (3 g)
- Ascorbic acid (3 g)
- Agar (10 g)
- The compounds are mixed in distilled water until 1000ml. For having a good homogeneity of the medium, this should be heated before autoclave (15 minutes)

The number of colonies in a viable count is made through spread plate method. A volume (0.1 ml) of an appropriate dilution pours out over the surface of the medium. The volume of the medium in Petri plate is around 15 ml. To avoid some errors, it is important to process the homogeneisation of the culture. The incubation is 37 deg C during (18-24 h).

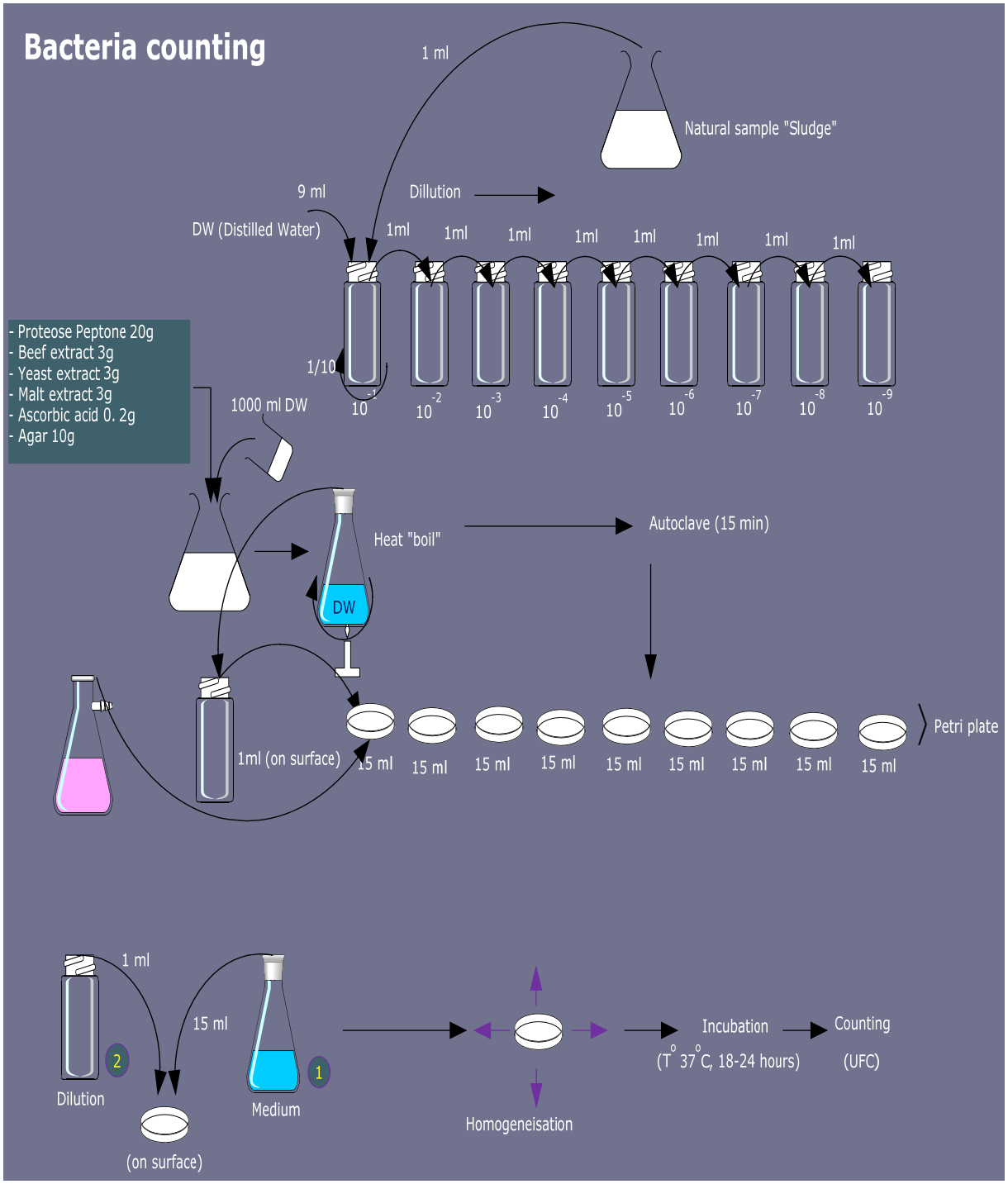


Figure 14: Bacteria counting

III. RESULTS AND DISCUSSIONS

III.1.1. Activities in the experiment period

The history of the experiment is shown by the figure below (Figure 15). This was demonstrated by a comparative study with a series of tests with different range of salinity. The experiment was evaluated by comparing a standard “without adding salt” and the one digestor on “adding salt”.

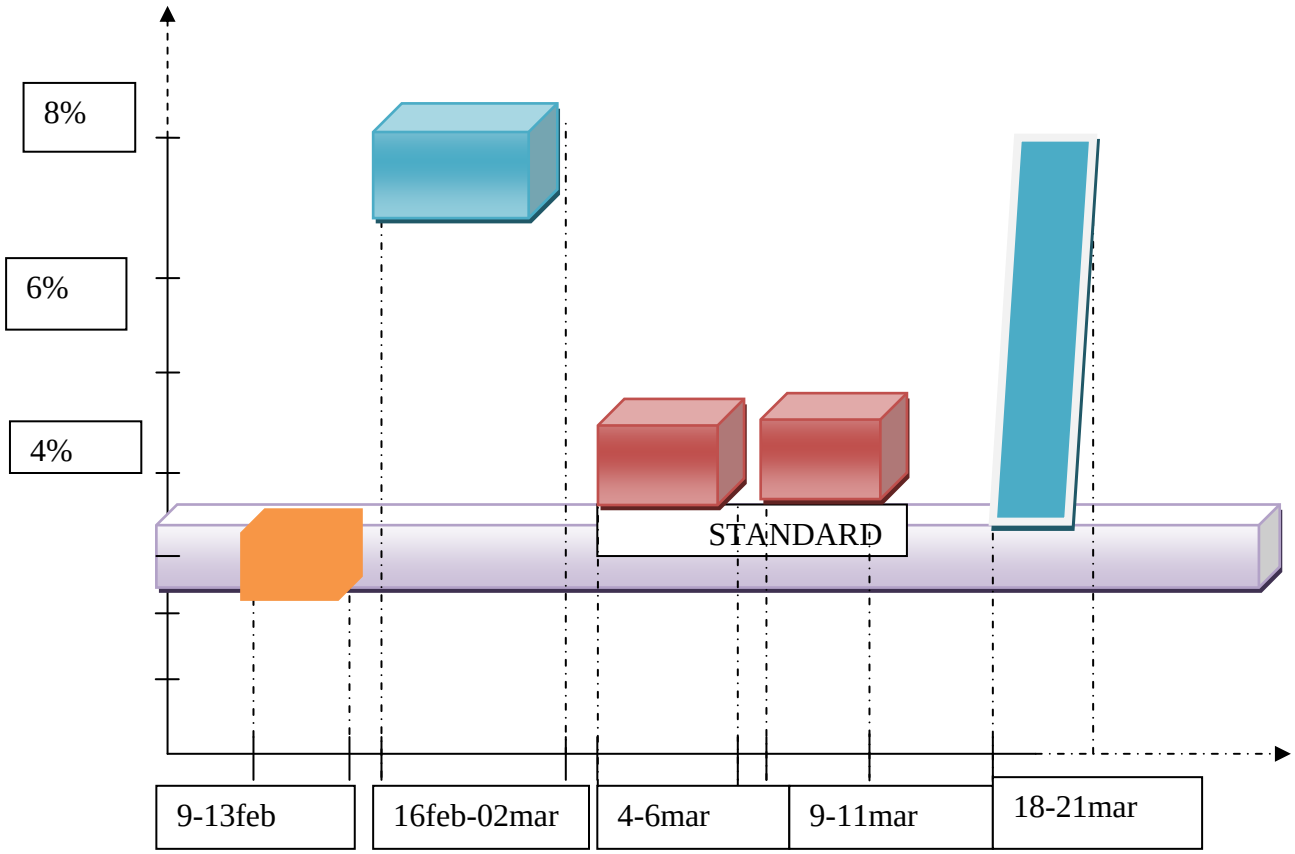


Figure 15: History of the experiment

III.1.2.Conditions of the experiment

All of the conditions of the experiment including temperature, pH, conductivity and salinity are presented on (Figure 16). Those curves describe the parameters considered along the experiment.

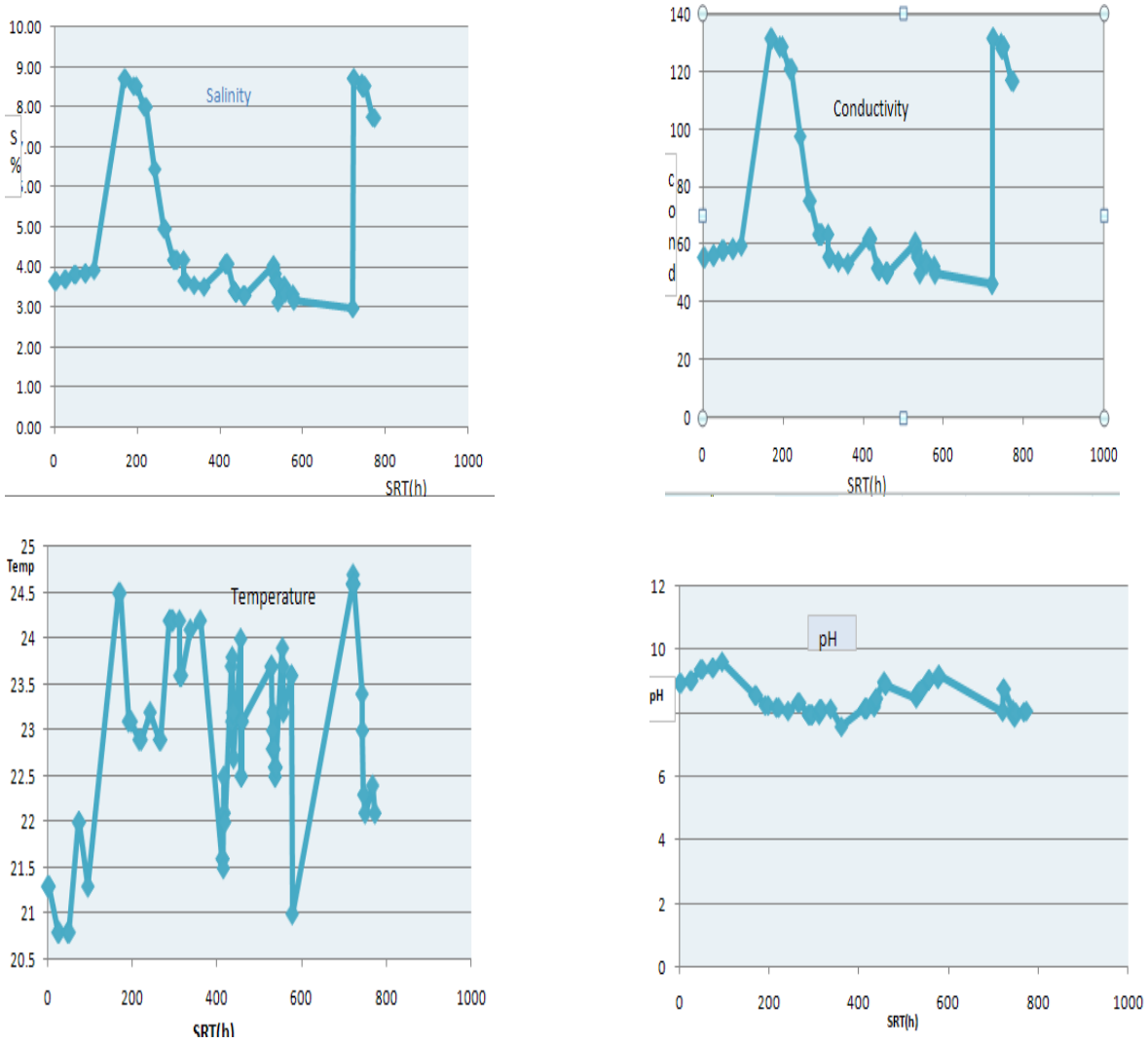


Figure 16: Conditions of the experiment

The temperature was relative stable during the experiment and was around 21-25 degC . The pH value varied between 8 and 9.2. The activated sludge process effectively operates over a pH range of 6.5 to 8.5. (Kenan, 1997). Neutralization may be required for wastewater that is outside of this pH range. If a complete mix activated sludge is used. Hydroxyl ions (OH-) will react with the carbon dioxide (CO_2) produced by microbial respiration yielding bicarbonate ion (HCO_3^-). This tends to buffer the system at a pH near 8. Organics acids will be biologically oxidized to CO_2 and water. The CO_2 will be air- stripped from the process to its equilibrium concentration and thus reduce the acidity.

For some type of wastewater in the digestor with a high salt concentration will produce alkalinity in the form of carbonate and cause the mixed liquour pH to increase to a range of 8.5 until 9.2. This may mislead that the salinity and pH are inter-dependent.

III.1.3. Relation between OUR and Conductivity

Different cure are obtained to show the relation between OUR and Conductivity. And they achieved from some cycle such as Cycle number 1 (09-11 February in (Figure 17) which reflect to the cycle without adding salt, cycle number 2 in (Figure 18) and Cycle number 3 (16-29 february) in (Figure 19) which is with high salt concentration(131.8mscm-^{1}).

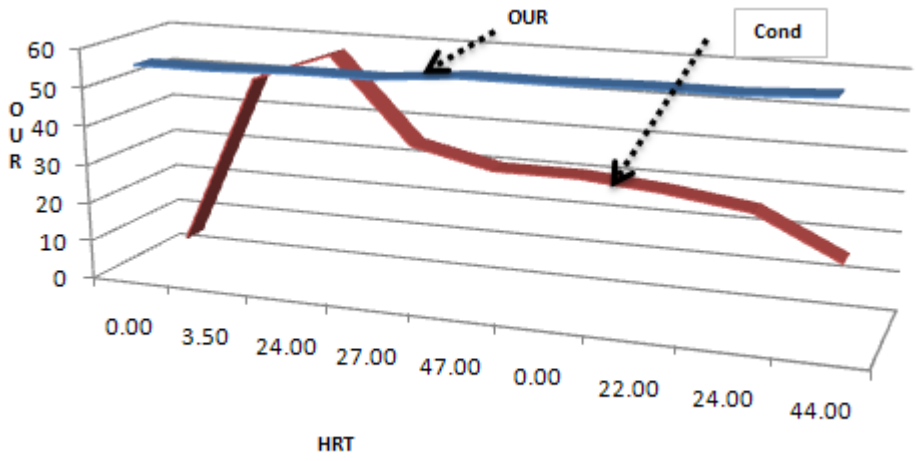


Figure 17: OUR and Conductivity (09-11 February)

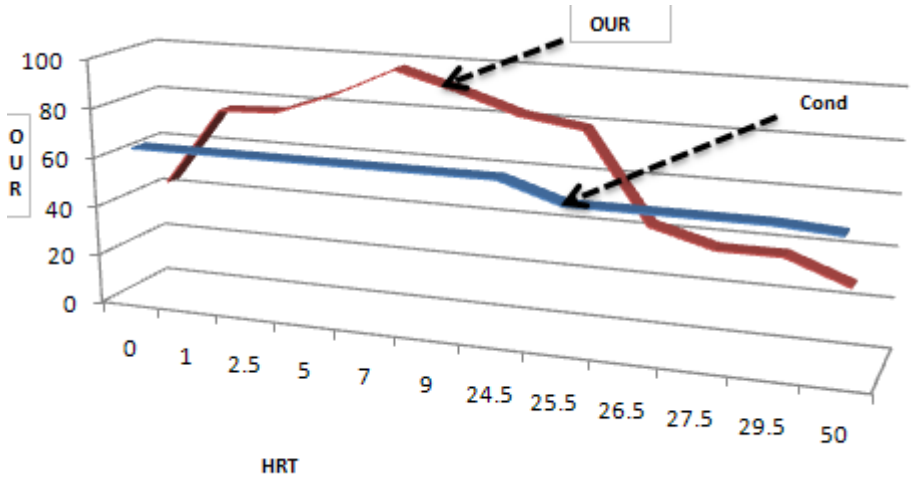


Figure 18: OUR and Conductivity (25- 02 mars)

The degradation of organic compounds in wastewater coincides with a rapid and significant rise of Oxygen Utilization Rate (OUR). After it reaches a plateau, OUR maintain a stable level a period and then to decrease until 24 hours for the cycle number 1 (09-11 February) and 25.5 hours for the cycle number 3 (25 February- 02 march).OUR attains the minimum level for the remaining period. These three distinctive phases of OUR are similar to the three phase of substrate degradation.

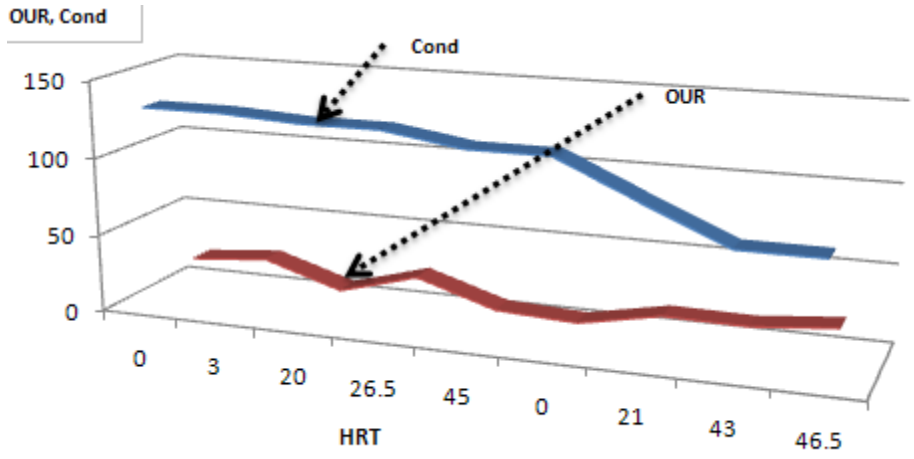


Figure 19: OUR and Conductivity (16- 23 February)

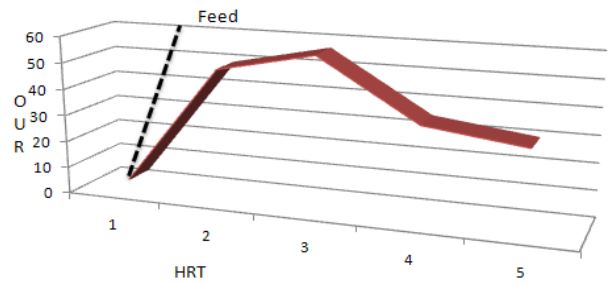
Considering the parameter of conductivity, the highest conductivity (131.8 mScm-1) is shown in (Figure 19). The phase of OUR is still low and constant along the cycle; from the beginning of the cycle (16 February) until the end (23 February). The shape of the curve is almost stable. At 3 hours it rises in a few amounts but in general, there is not variation. This implies that the biomass struggle to maintain their life at an inappropriate condition. Bacteria try to wrestle otherwise the high content of salt: Halophile bacteria and osmotolerant bacteria can survive. So the fact of this few increasing means the consumption of oxygen from those bacteria. And they are in minority that why it is just some amount of oxygen that they consume. The other types of bacteria may have died. They cannot grow in the condition. It should be noted that the bacteria containing in the sludge is a medium halophile as they are not so active on taking the oxygen during the cycle. Clearly, they tolerate the salt concentration around 3%. Extreme Halophile can tolerate high salt concentration until 15% such as *Halobacterium sp.*

III.1.4. Relation between OUR (oxygen Utilization Rate), TOC (Total organic Carbon) and VSS (Volatile Suspended Solids)

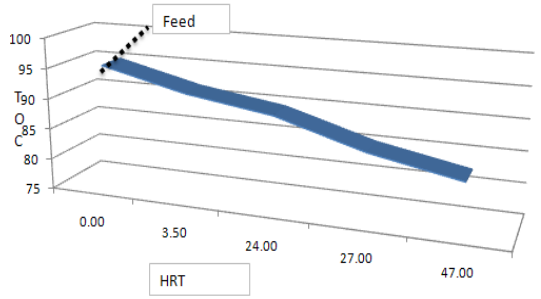
A typical example of one cycle in each series were observed with the relation existing between OUR, TOC and VSS (Figure 20) for cycle number"09-11 February", (Figure 21) for cycle number 2 "" , (Figure 22) for cycle number 3 and (Figure 23) for cycle number .

Cycle number 1 (09 Feb-11 Feb)

OUR



TOC



VSS

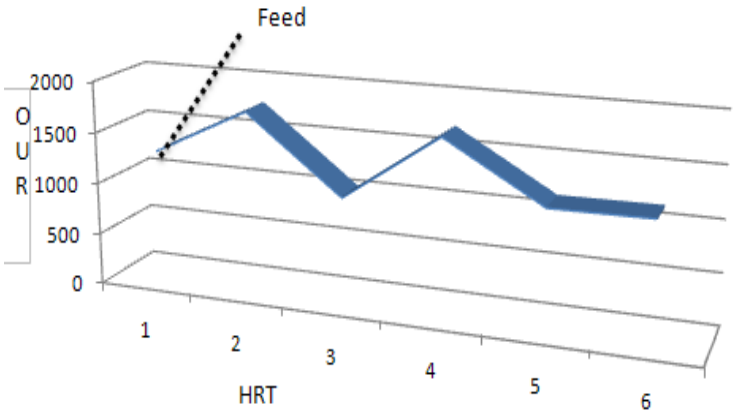
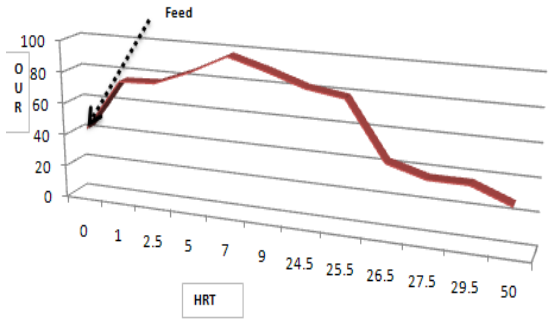


Figure 20: curve of OUR, TOC and VSS "Cycle number 1

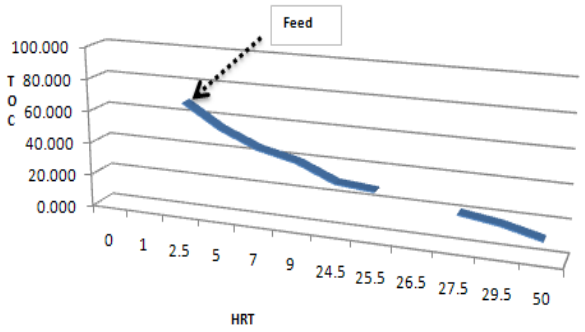
In the cycle number 3, there is a gradual removal of TOC (Figure 21) but not correspondingly increase in VSS due to analytical errors. The OUR is increasing as excepted, but variable and decreases towards the end of the cycle.

Cycle number 4 (25 Feb-02 mar)

OUR



TOC



VSS

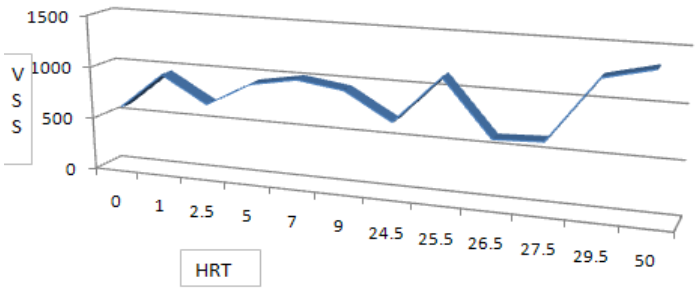


Figure 21: Curve of OUR, TOC, and VSS "cycle number 2"Cycle number 3

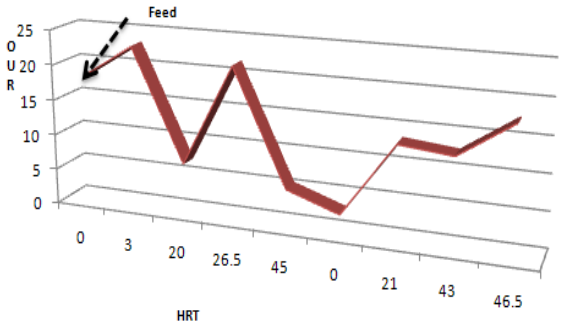
The development of OUR, biomass (VSS) and Substrate (TOC) during one cycle could be used to evaluate the growth process. In the beginning, substrate is in excess and the uptake is rapid. The salt added is partially penetrated and dissolved in the digestor. This mislead that the biomass is not yet affected .Some population try to synthesize those compounds for their growth which induce a rising the curve at 1-2 hours. It results in large increasing biomass (VSS), large consumption of oxygen (OUR) and large reduction of substrate (TOC). To summarize: the organic matter is removed from wastewater by biological metabolism, oxygen is consumed by the organism and new cell mass is synthetisized. The dying of biomass is seen by the reduction of VSS. That biomass is not resisting even a small amount of salt (Nacl). As

it is known the concentration of salt that we have in those cycles is around 4-3%. However, the halophiles bacteria are growing well and start to reproduce. This is shown in the cycle number two at 7 hours by increasing VSS. And they consumed OUR at maximum level with reduction of TOC.

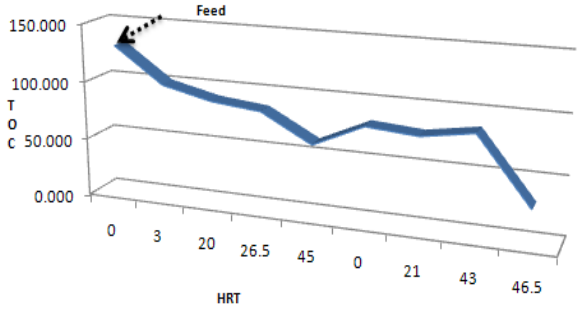
Once the total salt added are dissolved, the concentration of salt increase which make to reduce again VSS and OUR. At a certain period the substrate and the nutrient which support the growth are deficient. At time 27.5 hours in cycle number 3, we can observe that many microorganisms die after a relatively short time without substrate.

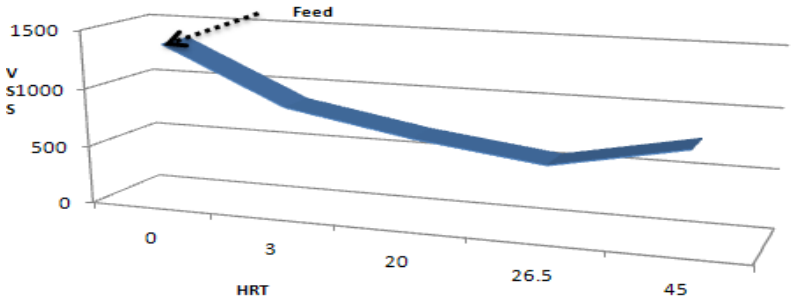
At the end of the cycle (t=29.5 h) the organism are in state of progression and process with the auto-oxidation "endogenous respiration" of their cellular mass. Bacteria precede the biomechanisms to survive by oxidizing the dead mass and nutrients.

OUR



TOC





VSS

Figure 22: link between OUR, TOC and VSS" Cycle number 3"

The maximum growth rates was determined in the cycle and is shown in (Figure 22) and (Figure 23) as a function of time and concentration. The remaining biomass after the cycle 1 and 2 are still alive. But they are in weak portion. The consumption is still low (18.36 mg/l) relatively to VSS (570 g). This deduces the non effectiveness of removal of substrate which is around 80 mg/l.

III.1.5. Kinetic growth rate

The analysis is based on calculating the kinetics of degradation "umax, Y and Kd".

- Umax is determined from the data by using the formula of Monod equation

$$u = u_{max} \frac{C}{C + K_s}$$ C: Limiting substrate (TOC)

VSS=X= Biomass

During the initial phase C>>>Ks ⟶ u=Umax

$$Y = \frac{\Delta X}{\Delta C} \rightarrow \frac{dC}{dt} = \frac{dX/dt}{Y} = \frac{u * X}{Y} \text{ and } (1-Y) = \frac{\Delta O}{\Delta C}$$

$$\frac{dO}{dt} = \frac{dC}{dt}(1-Y) \rightarrow \frac{u * X}{Y}(1-Y) \text{ and } \frac{dO}{dt} = OUR$$

$$OUR = \frac{u * X}{Y}(1-Y) \rightarrow u_{max} = \frac{OUR * Y}{X(1-Y)} \text{ and u=umax}$$

- Umax= maximum growth rate(1/d)
- U= specific growth rate(1/d)
- Ks= half saturation constant(mg/l)
- C= Growth limitng compound(mg/l)

- Y is the growth yield can be a correlation of $\frac{\Delta X}{\Delta C}$ and the decay rate. It is the ratio between biomass growth and substrate removal.

  (Aulie, 2007) determined the yield to 0.25 gVSS/gCOD . We can find:

  Y= 1.42 gCOD/gVSS*0.25gVSS/gCOD= 0.35

  The remaining energy is corresponds to in 1-Y and is oxidized in $(1-Y)=\frac{\Delta O}{\Delta C}$

The biomass fraction is assumed to be about 70% of VSS. Thus X_H= 0.7*VSS(Aulie, 2007). The table below had shown the value of kinetics degradation in the cycle 1, 2, 3, and 4. And the two following figure describe the substrate limited growth at a certain salinity.

HRT	Cond	OUR	VSS	XH	Y	1-Y	Umax
0	131.8	18.36	985	689.5	0.355	0.645	0.351737
3	131.8	23.04	985	689.5	0.355	0.645	0.441396
20	128.9	7.2	1084.2	758.94	0.355	0.645	0.125316
26.5	128.9	21.6	1084.2	758.94	0.355	0.645	0.375947
45	121.1	5.4	635.4	444.78	0.355	0.645	0.160372
0	121.1	2.88	635.4	444.78	0.355	0.645	0.085532
21	97.6	13.32	1048.6	734.02	0.355	0.645	0.239705
43	75.1	12.6	792.7	554.89	0.355	0.645	0.299946
46.5	75.1	17.64	792.7	554.89	0.355	0.645	0.419925
0	63.4	43.56	775	542.5	0.355	0.645	1.06064
1	63.4	75.6	775	542.5	0.355	0.645	1.84078
2.5	63.4	76.68	775	542.5	0.355	0.645	1.867077
5	63.4	85.68	815	570.5	0.355	0.645	1.983827
7	63.4	97.2	815	570.5	0.355	0.645	2.250559
9	63.4	90.36	976.6	683.62	0.355	0.645	1.745988
24.5	63.4	82.44	976.6	683.62	0.355	0.645	1.592954
25.5	55.5	78.84	913.2	639.24	0.355	0.645	1.629156
26.5	55.5	43.56	913.2	639.24	0.355	0.645	0.900127
27.5	55.5	36.72	913.2	639.24	0.355	0.645	0.758785
29.5	55.5	36.72	627.4	439.18	0.355	0.645	1.104435
50	53.9	27.36	1289	902.3	0.355	0.645	0.400539

Average max = 0.41 d^{-1}

Average max = 0.35 d^{-1}

Average max = 1.9 d^{-1}

Table 1: parameter of kinetics[OUR (mg/l), VSS(g),Cond($mScm^{-1}$), HRT(h), u_{max}(d^{-1})]

Organic compounds (TOC), oxygen and NH3 may limit the growth rate. The formula of Monod equation describes the growth rate affinity as a function of substrate concentration. This can make an analysis how easy or difficult the substrate utilize by bacteria. The easy compound refers to Ready biodegradable Compounds (RBCOD) and the difficult compounds refer to Slow Biodegradable Compounds (SBCOD).

Observing the two figures below (Figure 23) and (figure 24), the growth rate is low in the beginning of the experiment. This is because of the bacteria is acclimating to the new substrate in the wastewater. They are not stable to oxidize the compounds. The experiment reaches three cycles, bacteria become more comfortable by adapting to the salinity and they are able to utilize the substrate for their metabolism and growth.

The growth rate is linked to the substrate. For this case, we can find that the value of K_s is around 54.080mg/l. This is an average value if we evaluate the range value of TOC. That means wastewater from oil industry contain mixing compounds with SBCOD and RBCOD.

It should be mentioned that K_s measure the affinity of the substrate and the affinity of specific organisms to substrate. RBCOD correspond to small simple molecule which have a high affinity to organisms and easy to uptake by the bacteria. For those large and complex molecule may be converted to small molecule before diffusing and transported to the internal microorganisms.

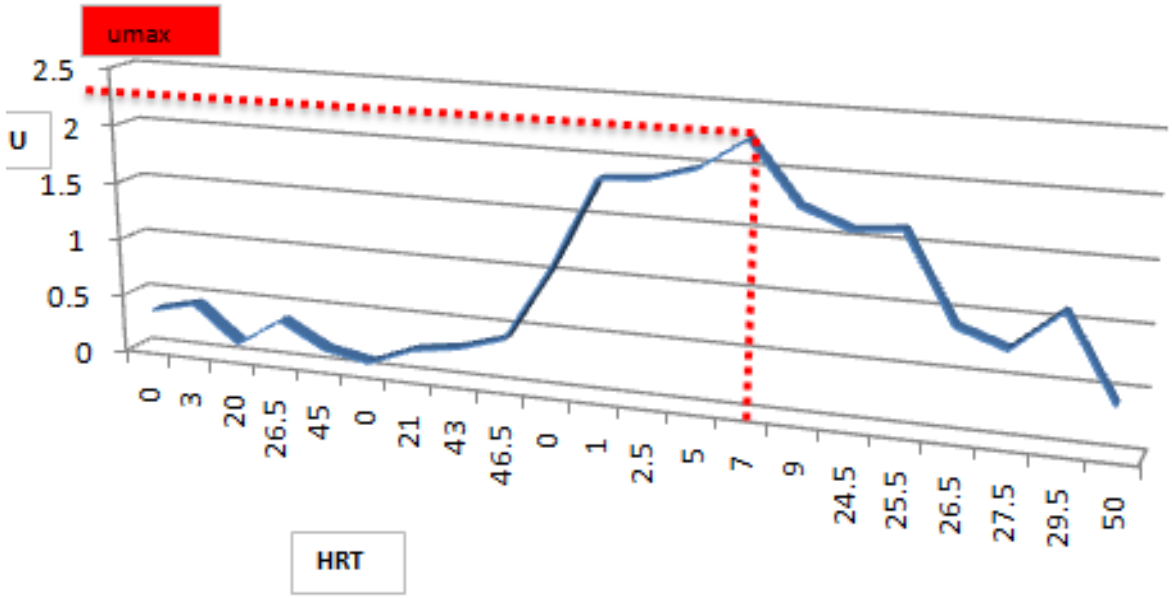


Figure 23: Substrate limited growth as function of operator time [HRT (h), u (d^{-1})]

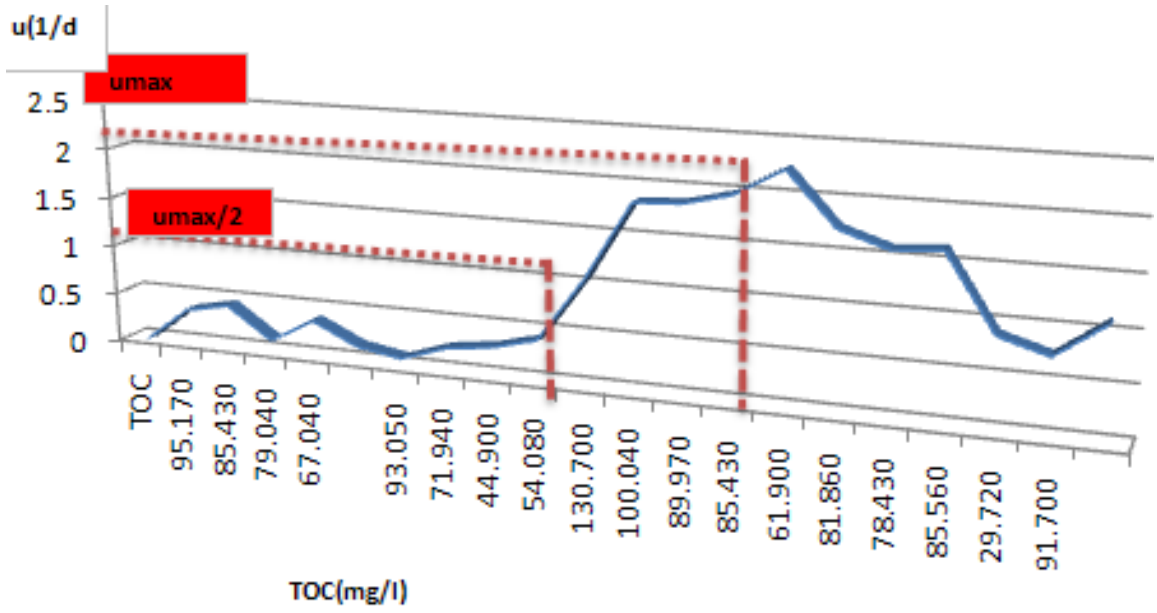


Figure 24: Substrate limited growth as a function of substrate Concentration

III.1.6. TSS and VSS

TSS and VSS during the experimental eriod is shown in(Figure 25) and (Figure 26). The VSS is about 20-30% of TSS and the large inorganic fraction is mainly related to recitation of salt during the test.

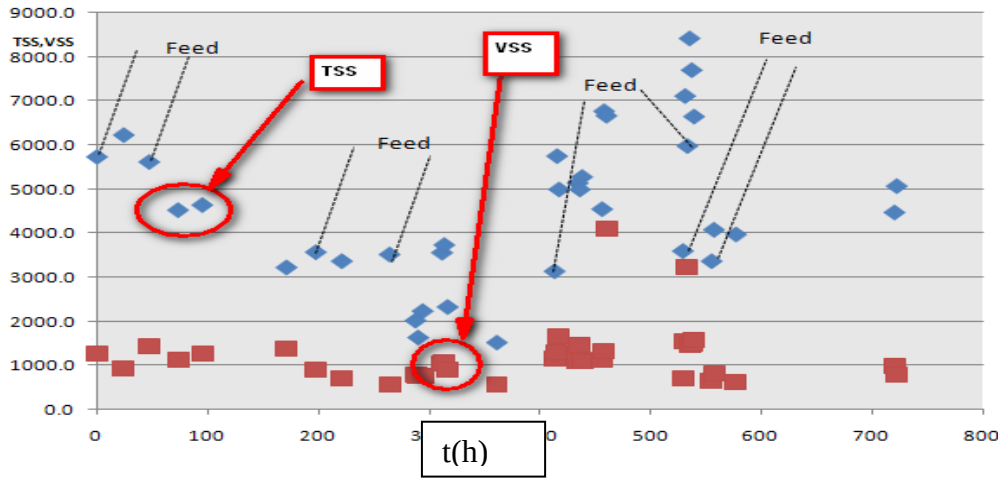


Figure 25: Curve of TSS and VSS in adduction of salinity

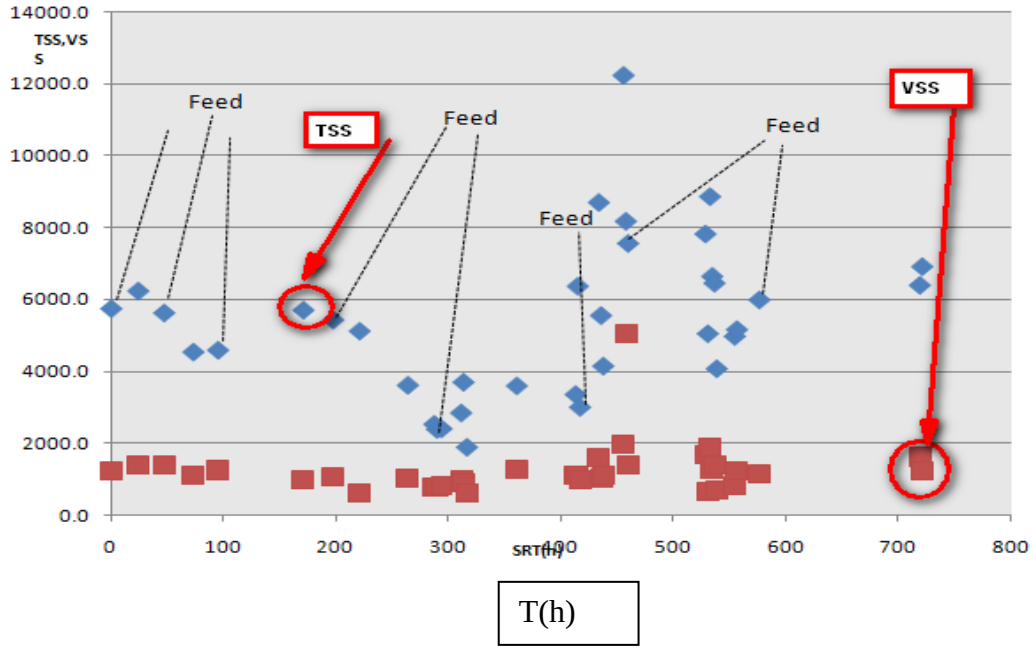


Figure 26: Curve of TSS and VSS without adduction of salinity

IV. MICROBIOLOGICAL INVESTIGATIONS

IV. 1. Fresh microscopy observation of the sludge

i. Cycle 1 "131.8- 121.1 mScm-1":

The fresh microscopy observation is doing through the historical of the experiment. This allows us to understand easily how incorporate the biomass population in the sludge. (Figure 27) shows that the picture of floc in 100 um when the adduction of salt"131.8 mScm-1 is applied. The period of experiment was in the 16 of February until 23 February 2009. From that picture, we can see some trace of dead cell membrane which looks like a little shoot line. And some viable bacteria can be detected closed to the deposit. They are easily recognized trough their gilded properties. This makes it imperatives that biomass are in state of doing endogenous respiration. They utilized the cell materials for the generation with the nutritional and environmental conditions. It could be taken from this observation that the bacteria resisting in this condition is a halotolerate and osmotolerate bacteria. Under such circumstances, any protozoa do not find in the sludge.

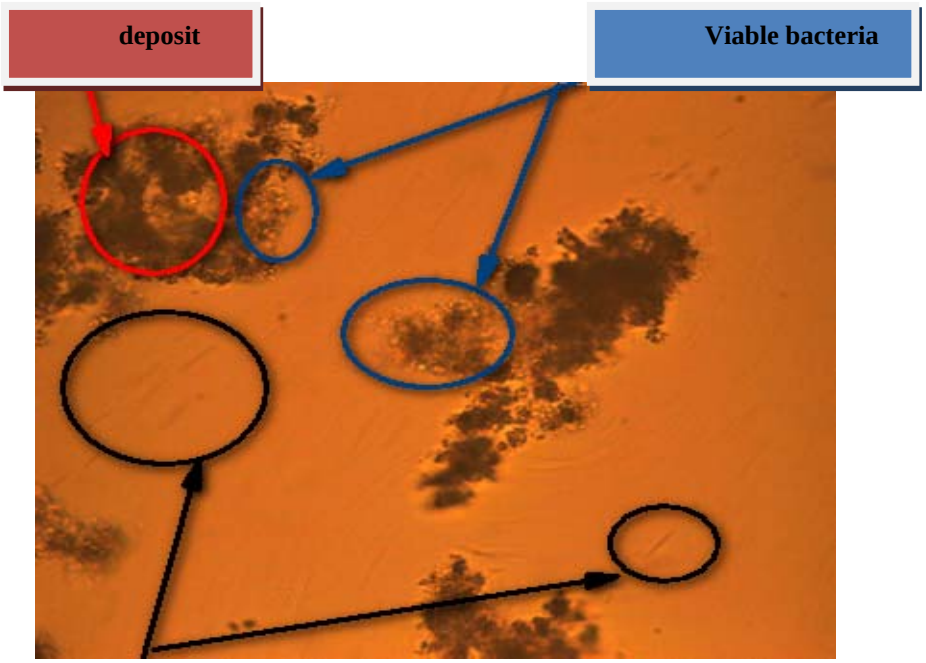


Figure 27 : sludge in 131.8mScm-1

ii. Cycle 3"63.4-53.9 mScm-1":

As a result of fresh observation of the sludge, the picture below (Figure 28) and (Figure 29) reflect the image in general at what constitute the sludge in the conductivity of "63.4-53.9 mScm-1".Sludge was taken during the cycle number 3 (25 February until 02 march 2009).

- **Observation of Floc:**

This consists on observing the large flocs sinked to the bottom of the digestor. The floc is approximately in size of 0.5 um. According to these pictures (Figure 28) many microorganisms are living and containing in the sludge including bacteria and protozoa. Some deposit constituted the floc. It could be a particulate organic or inorganic or inert. In this case, it is shown that numerous protozoa are found. And the latter are catching beside the deposit. On comparing to the publication (Jenkins et al., 2003), those protozoa correspond respectively to *Paramecium sp*, *Epistylis sp*. and Bacteria. The references picture (Figure 31), (Figure 33), (Figure 35) and (Figure 37) are shown respectively closed the species found (Figure 30), (Figure 32), (Figure 34) and (Figure 36).

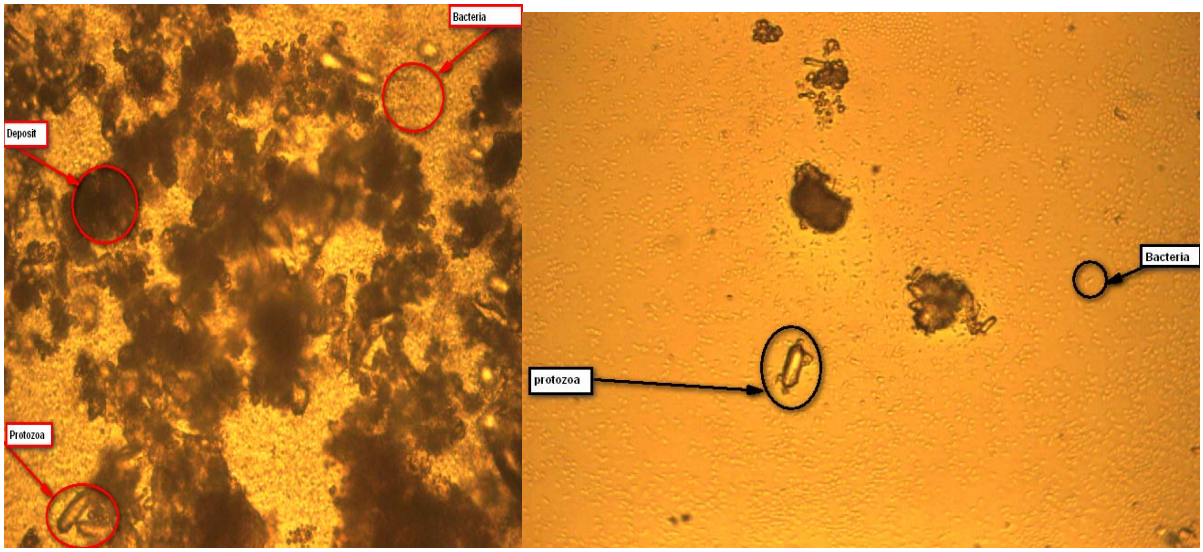


Figure 28: fresh observation of the sludge

- *Paramecium sp.*:

Paramecium sp. is a group of unicellular ciliate protozoa which have a range about 50um in length. The taxonomy is:

-Domain :Eukaryota

-Kingdom: Protista

-Phylum: Ciliphora

-Class: Ciliatea

-Order: Peniculida

-family: Parameciidea

-Genus: Paramecia (lekomtsev et al. 2007)

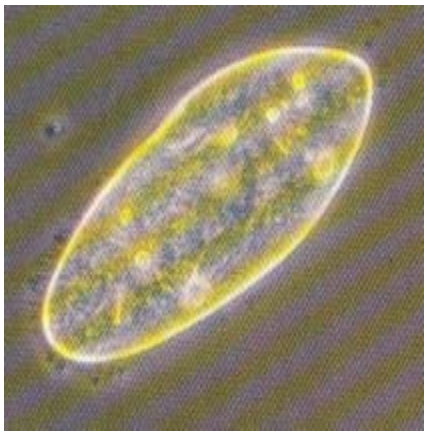


Figure 30: *Paramecium sp.* **Figure 31: Phase contrast micrograph of *Paramecium***

The body is cover of cilia which allow the cell to move. This species is equipped by a pair of contractile vacuoles which allow it to accomplish the osmoregulation (Aury et al., 2006). That apparatus makes the Paramecium to resist in the environment with high concentration of salt.

They are also present in sludge as predator. As it is known, this ciliate feed on microorganisms like bacteria, algae….

- *Epilitylis sp.*:

It should be précised that *Epistylis sp*. like to live in group. On enlarging some section of the sample, it can be noted this protozoa live together near their generation (Figure 32) which makes indicate that they are immobile. Clearly, the fact that the generation is found just closed the parents' protozoa means and describes non displacement of the microorganism. They do not have any locomotion equipment to accomplish this act. They are in state of growing. Many *Epilistylis sp*. daughter leave closed the parents (Figure 32). This is because of the stabilization of the condition.

Figure 32: *Epilitylis sp.*

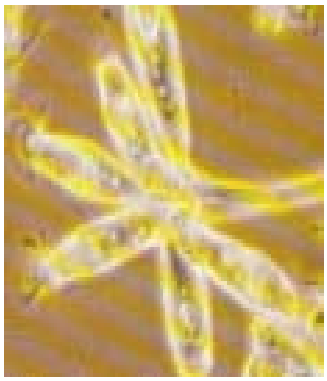


Figure 33: Phase contrast micrograph of *Epilitylis*

- Bacteria:

In addition, if some part of the sample is analyzed, it was detected that bacteria is in forms of coccus. The historical of experiment brings a good explanation. A we can see the experiment is already affected on adding high salt concentration before it comes down on cycle number 3. As already indicated the treatment start from 131.8 mScm-1 or in salinity 8% on cycle number 1 and arrive in conductivity is 63.4 mS cm $^{-1}$ until 53.6 mScm^{-1}or in salinity (4-3%) on cycle number 3. This mislead that this range is suitable for the growth of bacteria and making the biomass start to get volume. This make recognized by the detection of bacteria

during microscopic observation. The delimitation of the bacteria membrane helps to find them easily. The sample is taken from the beginning of the cycle number 3. Substrate and nutrients are sufficient to be taken into the cells. Both the size and the mass of bacteria increase. In (Figure) is carried out exclusively image to make an evidence how bacteria community looks like in the sludge at cycle number 3. They are not yet in community but they leave individually. This is the period when growth rate start to increase simply the lag phase of this cycle. A portion of this figure shows a rough drawing of some cells in state of dying and reproduction. The shoot line represents the dead bacteria which do not resist the condition before (131.8mScm-1). Also it saw on this latter figure that some bacteria cells begin to divide (Figure). This implies that a sufficient concentration of substrate and appropriate condition (salinity around 4-3%) make them to build up. And it will result after this phase a rapid increase in the number and mass of microorganisms. This is a period when the substrate conversion is at its maximum rate.

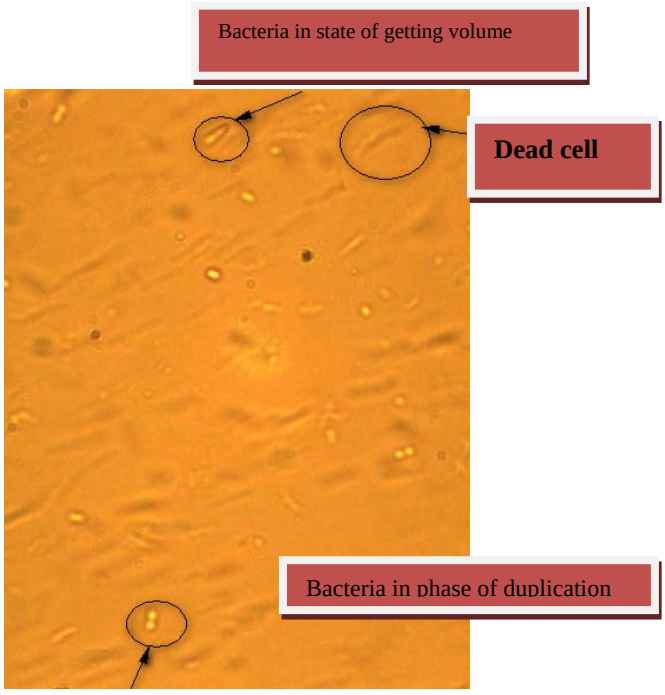


Figure 34: Bacteria containing in the sludge

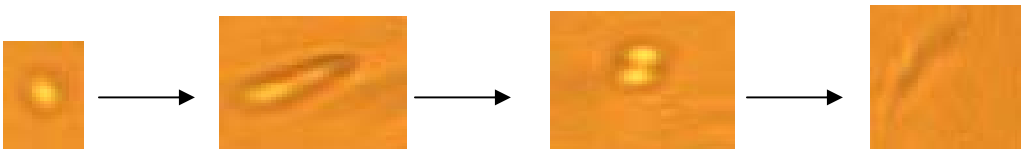


Figure 35: Life cycle of Bacteria

It was detected in (Figure 34) some bacteria in state of copy a daughter bacteria, some in state of elongation and some are die.

Since the salt concentration constitutes a powerful selective influence of the sludge ecosystem, fewer species have been identified. The habitat of this sludge is halopihilic organisms which grow in the environment in sodium chloride at concentration" ". The lower limit varies slightly with genus "*Halobacterium* and *Halococcus*" with nutritional conditions. (Brown, 1976). By far and away, it is crucial to reveal from the picture that bacteria represent the 80% of biomass containing in the sludge. For the evaluation the efficiency of the treatment on using activated sludge, bacteria should be controlled on priority in terms of growth, biology, conditions…..

- **Observation of the water floc :**

The species found in the intertidal water which is the accompanied with floc, do not contain so many organisms. Comparing to the picture, the habitat were identified by *Uronema sp*. and copepode (Jenkins, 2003). Note that it is just their dead cells that were observed to the microscopy. It was recognized because copepode should be a mobile microorganism. But we are finding it in a stationary position.

- Uronema sp.:

It is pathogenic marine ciliate. And the growth for Uronem sp. And bacteria illustrated typical predator-prey relationship. Note that this is surviving specie after the adduction of high salt concentration. This induce that this specie is present in the sludge when the experiment started. But because of the high salinity, most of them died. This means that this ciliate feed bacteria. It is important to mention that this species is vulnerable to salinities lower than 3.5

ppt (Novotny, 1996). And they disappear under high salinity conditions. Uronema sp. is bacteriovorous. Bacteria become available in the environment but the abiotic factors which include the condition of experiment do not make them in a good motility to search food.

- Copepodes:

This zooplankton is a small crustacean in sea. This induce that this species is brought with wastewater but not contained in the sludge. It has a teadrop shaped body and large antenna (Marten and Reid, 2007). In (Figure 36) the antenna is missed as it is already a dead microorganism.

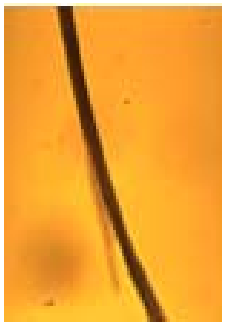


Figure36: *Uronema sp.*

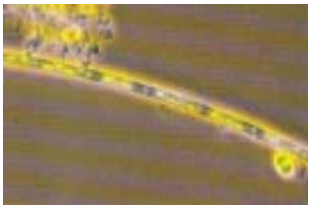


Figure 37: Phase contrast micrograph of *Uronema*

Herbivorous Copepods feed directly the smaller species such as phytoplankton. Benthic copepods eat organic detritus or bacteria (Vu, 2005). This crustacean takes oil droplets as a food in rich cold seas. They feed in the spring and summer plankton bloom (Anonym, 2006). This indicates that it is bringing with wastewater from the reservoir and transport to the digestor to participate on the treatment.


Figure38: Copepodes

- **Observation of the effluent :**

We can conclude the efficiency of the treatment on observing the effluent by microscopy. In this instance, there are no microorganisms living in the effluent. And it is just the deposit (figure 39) that contain in the sample. This could be a particulate compound.

Figure 39: Effluent obtained after cycle 3

IV. 2. Sludge community

i. Predator-prey relationship:

The Copepodes, *Paramecium sp.*… are the predation of bacteria. They interact with bacteria habitat to consume this specie as their food. The predator cited above will die if it

does not get food, so it evolves whatever is necessary to eat the prey. The use their system of locomotion, prey catching to uptake it. For instance, Paramecium sp. used ciliate to take bacteria. The presence of such species is necessary and indicates a good efficiency of the treatment. As it is known, they maintain the equilibrium of the ecosystem. This relationship is a good steady state for the biocenose in sludge.

ii. Host interaction:

The sludge habitat use wastewater as their host for living. And the content of wastewater is their source of energy for their generation.. Bacteria oxidize the organic compound in wastewater, while Copepode feed oil droplet and bacteria. And Paramecium sp. eats bacteria too. It is important to mention that most of the content of bacteria body is water.

iii. The food chain trophic:

The sludge community can form one ecosystem. And the biocenose is: Paramecium sp., Epilitylis sp., Copepodes a Bacteria…The food c hain trophic between the biocenose are represented below. This related the interaction for each over in terms of nutrition.

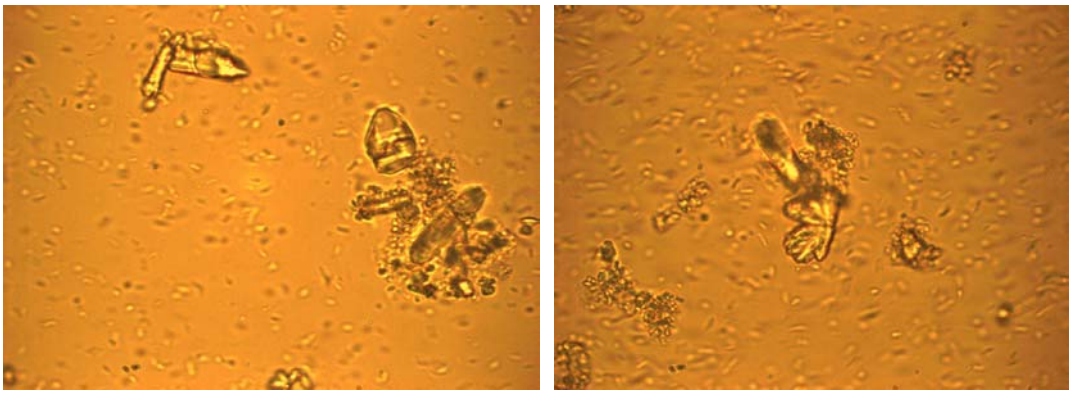


Figure 40: Biocenose in sludge

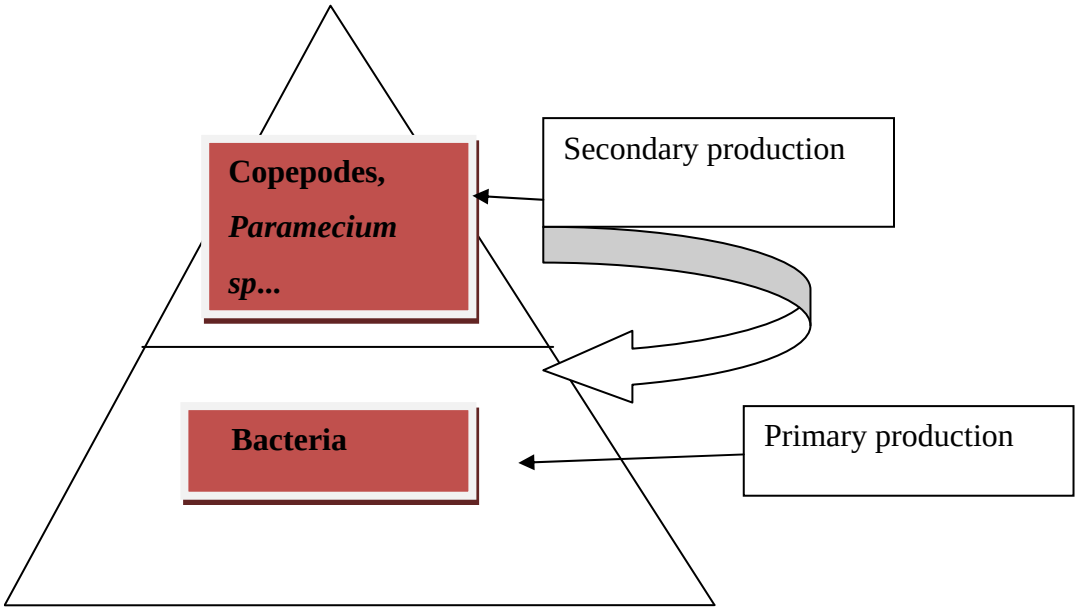


Figure 41: Food chain trophic

iv. Competion :

The secondary production could be on competition of food supply. And they use their rapid motility, swarming behavior permit them to catch easily the prey.

IV.3. Bacteria identification and quantification:

- Form this picture, we can define that the bacteria is gram positive as it is colored in pink. This makes confirm that they are Gram positive. This kind of has got higher amount of peptidoglycan compared to gram negatives. This peptoglycan is the responsible for retaining the crystal violet during gram staining. And it is the cell wall and membrane which is very thick in gram positive comparing to negative. The gram positive are stained with crystal violet. The gram negative are also stained by crystal violet but this stain is removed by washing it with 95% alcohol This is due to the presence of lipids in cell wall equipped by pores and make let the stain to escape. And the safari gives the ink color. In Cycle number 1 before decantation, the species bacteria is not yet identified but the characteristics and morpholology are already described by following the methods

microscopy observation. According to the picture obtained (Figure 42), the bacteria is in form of coccus living in cluster. On continuing the research in cycle number 3 with conductivity “63.9 mScm-1”, we can reveal that it is *Staphylococcus aureus* the sampling was done just after feeding. The taxonomy is (Anonym, 2006) :

-Kingdom: Bacteria

-Phylum: Firmicutes

-Class: Bacilli

-Order: Bacillales

-Family: Staphylococcaceae

-Genus: Staphylococcus

-Species: aureus

They are in form nearly spherical and facultative anaerobes. Also, they resist to desiccation and high osmotic pressure.

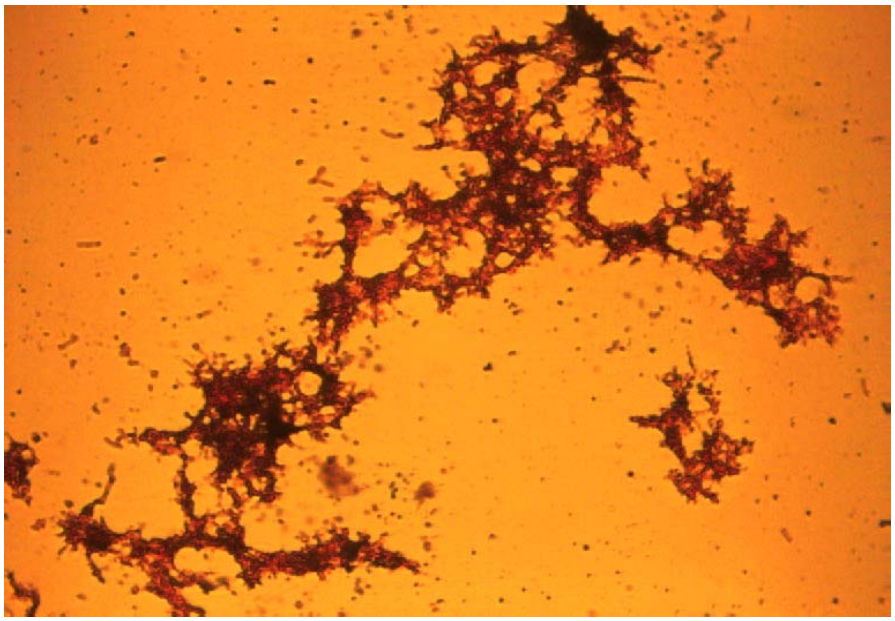


Figure 42: Gram staining in cycle 1 with 131.8 mScm-1 before decantation

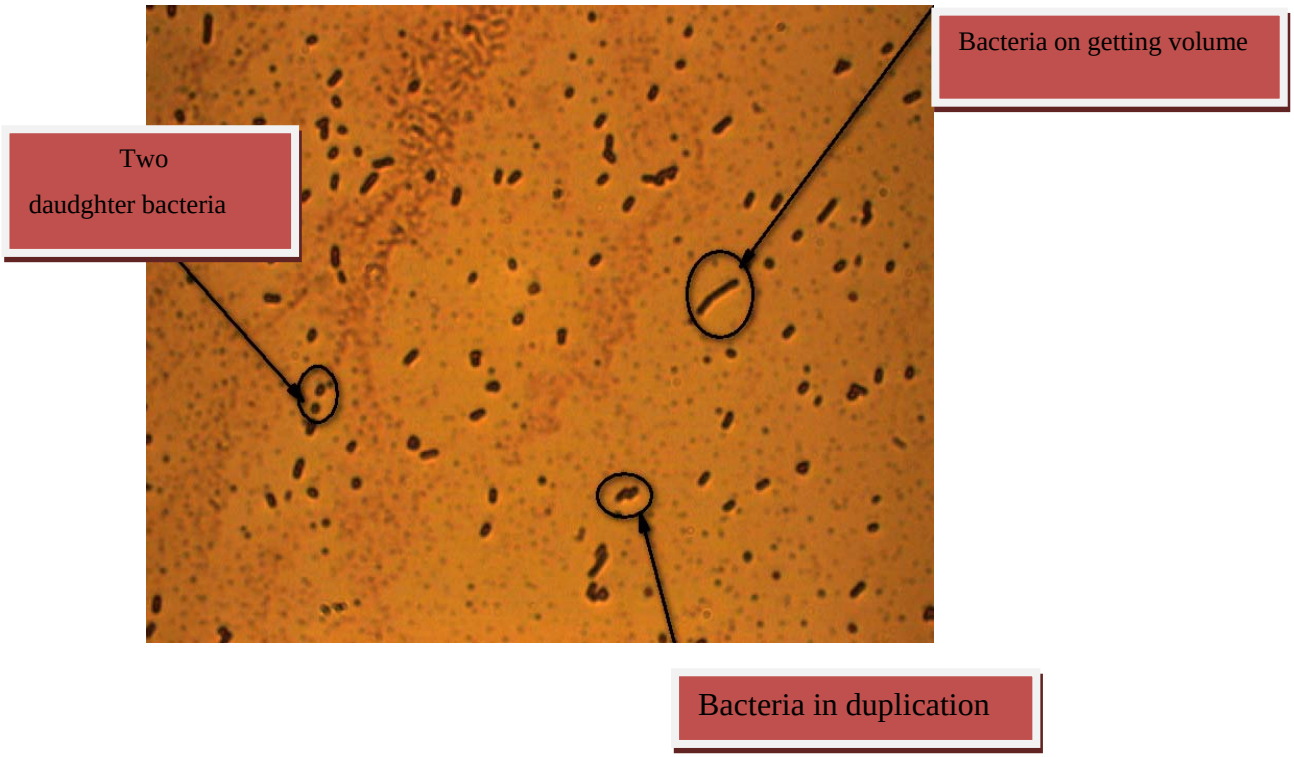


Figure43: Gram staining in cycle 3 after feeding.

We can detect some bacteria in state of taking a weight. And when they attend the sufficient a volume (Figure 43). The cell elongates and separates into two strands. Finally, the cell membrane grows inward. Bacteria start to split and duplicate into cell daughter. They are among the animal that grow faster. It can make a million of copies of itself in the right environment in 24 hours.

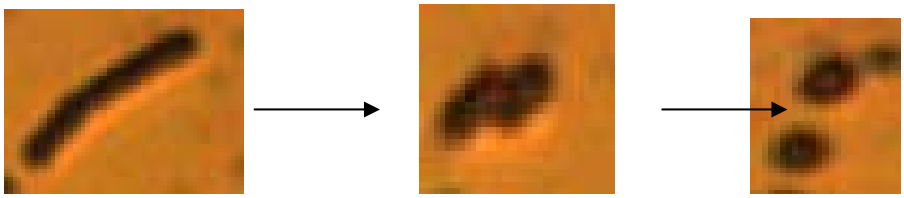


Figure 44: Life cycle of Bacteria

CONCLUSION

Production and optimization of biological wastewater treatment from oil industry is controlled be several factors such as substrate, nutrient, salinity….The local conditions and the requirement dictate the operators to treat those streams. This tends the oil and gas industry to move toward the zero discharges.

Biological treatment using activated sludge is concluded as a most efficient when we are looking on the cost- effectiveness. It is cheap and the degradation of compounds containing in the wastewater removed in approximately 90 %.

Growth response of biomass population was study in different range of salinity. This is for evaluating the effect of salinity in biological wastewater treatment for oil industry. Clearly, the variation of salinity which were investigated to gather fundamental information on environmental parameters the oxidation of the compounds content in wastewater.

There are many methods used to accomplish this evaluation like measure of parameters, phosphorus, T0C analysis, VSS and microbiological investigation. Those control permit to establish the real effect of salinity.

The salinity in the range 4-3% gives good effectiveness on removal the substrate"54.080 mg/l" with oxygen consumption "97.2 mg/l", umax "2.5 d^{-1}". And the VSS is 815 g. However, for salinity 8% the Oxygen Utilization Rate is 18.36 mg/l, umax "0.35 d^{-1}" and VSS is 985 g.

The salt adduction is associated with a very distinctive ecology. This constitutes a powerful selective influence on the sludge community. This factor can define and qualify the genera able to survive in this condition. According to the microbiological result, the bacteria found are gram positive, in form of coccus and live on clusters. From this information, we can deduce that this is *Staphylococcus aureus*.

This species needs salt for their metabolisms and this is for maintaining the structural integrity of the cell envelop. This makes the population to dominate in this sludge. . The intracellular of staphylococcus “cytoplasm” is accumulated by a massive of KCl (Potassium Chloride) and effective exclusion of sodium (Na^+).

The instability of cell envelop during the adduction of salt for nonhalophiles bacteria reflects non support of the condition. This leads the death of some bacteria. Sodium Chloride brings the u to normal strength. In response, the membrane becomes weak and disaggregates the cell.

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APPENDIX 1 :

APENDIX 2 :

t(d)	t(h)	Date	hours	HRT	Temp	pH	Cond	S%	OUR
1	0	9-Feb	9:00	0.00	21.3	8.96	55.4	3.66	3.96
	3.5	(feed)	12:30	3.50	21.3	8.96	55.4	3.66	49.32
2	24	10-Feb	9:00	24.00	20.8	9.04	56.1	3.71	56.88
	27		12:00	27.00	20.8	9.04	56.1	3.71	34.92
3	47	11-Feb	8:00	47.00	20.8	9.4	57.9	3.83	30.24
	51	(feed)	12:00	0.00	20.8	9.4	57.9	3.83	30.24
4	73	12-Feb	10:00	22.00	22	9.45	58.4	3.86	28.44
	75		12:00	24.00	22	9.45	58.4	3.86	25.56
5	95	13-Feb	8:00	44.00	21.3	9.64	59.5	3.93	15.12
8	168.00	16-Feb	9:00	0	24.5	8.57	131.8	8.72	18.36
	171.00	(feed)	12:00	3	24.5	8.57	131.8	8.72	23.04
9	191.00	17-Feb	8:00	20	23.1	8.25	128.9	8.52	7.2
	197.50		14:30	26.5	23.1	8.25	128.9	8.52	77.76
11	216.00	19-Feb	9:00	45	22.9	8.18	121.1	8.01	5.4
	221.00	(feed)	14:00	0	22.9	8.18	121.1	8.01	2.88
12	242.00	20-Feb	11:00	21	23.2	8.07	97.6	6.45	13.32
15	264.00	23-Feb	9:00	43	22.9	8.34	75.1	4.97	12.6
	267.50		12:30	46.5	22.9	8.34	75.1	4.97	17.64

Table 2: Data of cycle number 1 -2 with add salinity

APPENDIX 3 :

t(d)	t(h)	Date	hours	HRT(h)	Temp(deg	pH	Cond(mSc	S%	OUR(mg/l)
17	287.50	25-Feb	8:30	0	24.2	7.95	63.4	4.19	43.56
	288.50	(feed)	9:30	1	24.2	7.95	63.4	4.19	75.6
	290.00		11:00	2.5	24.2	7.95	63.4	4.19	76.68
	292.50		13:30	5	24.2	7.95	63.4	4.19	85.68
	294.00		15:00	7	24.2	7.95	63.4	4.19	97.2
	296.00		17:00	9	24.2	7.95	63.4	4.19	90.36
18	311.50	26-Feb	8:30	24.5	24.2	7.95	63.4	4.19	82.44
	312.50		9:30	25.5	23.6	8.12	55.5	3.67	78.84
	313.50		10:30	26.5	23.6	8.12	55.5	3.67	43.56
	314.50		11:30	27.5	23.6	8.12	55.5	3.67	36.72
	316.50		13:30	29.5	23.6	8.12	55.5	3.67	36.72
19	**337.00**	**27-Feb**	**10:00**	50	24.1	8.14	53.9	3.56	27.36

Table 3: data from cycle number 3 with add salinity

APPENDIX 4:

t(d)	t(h)	Date	hours	HRT(h)	Temp(deg	pH	Cond(mSc	S%	OUR(mg/
22	361.00	2-Mar	10:00	0	**24.2**	**7.57**	**53.2**	3.52	27.36
24	412.25	4-Mar	13:15	0hrs	21.6	8.13	61.9	4.09	54.8
	413.25		14:15	1hrs	21.6	8.14	61.9	4.09	89.6
	414.25		15:15	2hrs	21.6	8.18	61.6	4.07	34.2
	415.25		16:15	3hrs	21.5	8.14	62.1	4.11	23.4
	416.25		17:15	4rs	22.5	8.14	62.2	4.11	31.1
	417.25		18:15	5hrs	22.1	8.14	62.2	4.11	67.6
	418.25		19:15	6hrs	22	8.16	61.8	4.09	77.2
25	434	5-Mar	11:00	31hrs	23.7	8.2	51.7	3.42	43.5
	435		12:00	32hrs	23.1	8.31	51.7	3.42	55.5
	436		13:00	33hrs	23.8	8.41	51.6	3.41	66.9
	437		14:00	34hrs	**23.2**	**8.44**	**51.2**	3.39	**65.8**
	438		15:00	35hrs	23.2	8.46	51.1	3.38	98.7
	439		16:00	36hrs	22.7	8.48	51	3.37	32.4
26	456	6-Mar	9:00	42hrs	24	9.01	50.2	3.32	123.1
	457		10:00	43hrs	22.5	8.95	50	3.31	65.1
	458		11:00	44hrs	22.5	8.94	49.9	3.30	23.4
	459		12:00	45hrs	23.1	8.9	50	3.31	47.7
	460		13:00	46hrs	23.1	8.9	50	3.31	12.3

Table 4: Data from cycle number 4 with add salinity

APPENDIX 5:

t(d)	t(h)	Date	hours	HRT(h)	Temp(deg	pH	Cond(mSc	S%
29	528	9-Mar	9:00	0	23.7	8.48	60.3	4.04
	529		10:00	1	23.7	8.58	60.7	4.07
	530		11:00	2	23.7	8.61	59.5	3.98
	531		12:00	3	22.8	8.61	57.7	3.85
	532		13:00	4	23	8.61	57.9	3.85
	533		14:00	5	23.2	8.68	57.9	3.85
	534		15:00	6	22.8	8.66	55.8	3.69
	535		16:00	7	22.8	8.67	55.5	3.67
	536		17:00	8	22.6	8.68	55.1	3.67
	537		18:00	9	22.5	8.7	54.9	3.64
	538		19:00	10	**22.5**	**8.71**	**54.8**	3.62
	539		20:00	11	22.6	8.75	54.8	3.62
	540		21:00	12	22.6	8.75	49.8	3.14
30	555	10-Mar	12:00	27	23.9	9.05	54.2	3.56
	556		13:00	28	23.7	9.06	54.2	3.51
	557		14:00	29	23.2	9.08	53.1	3.36
31	576	11-Mar	9:00	48	23.6	9.1	52.3	3.34
	577		10:00	49	23.6	9.12	51	3.26
	578		11:00	50	21	9.23	49.7	3.17

Table 5: Data from cycle number 5 with add salinity

APPENDIX 6:

t(d)	t(h)	Date	hours	HRT(h)	Temp(deg	pH	Cond(mSc	S%
38	720	18-Mar	9:00	0	24.6	8.07	46.2	2.99
	721		10:00	1	24.6	8.07	46.2	2.99
	722		11:00	2	24.7	8.78	131.8	8.72
	723		12:00	3	24.6	8.78	131.9	8.72
	724		13:00	4	24.6	8.77	131.8	8.72
39	743	19-Mar	8:00	15	23.4	8.11	130	8.60
	744		9:00	16	23	7.94	128.4	8.49
	747		12:00	19	22.3	7.87	128.2	8.48
	750		15:00	21	22.1	8.02	129	8.53
40	768	20-Mar	9:00	39	22.4	8.04	117.3	7.76
	773.5		14:30	45	**22.1**	**8.07**	**116.9**	7.73

Table 6: Data from salt shock with add salinity

APPENDIX 7:

SRT(d)	SRT(h)	Date	hours	HRT	Temp	pH	Cond	S%
1	0	9-Feb	9:00	0	21.3	8.96	55.4	3.66
	3.5	(feed)	12:30	4	21.3	8.96	55.4	3.66
2	24	10-Feb	9:00	24	20.8	9.04	56.1	3.71
	27		12:00	27	20.8	9.04	56.1	3.71
3	47	11-Feb	8:00	47	20.8	9.4	57.9	3.83
	51	(feed)	12:00	0	20.8	9.4	57.9	3.83
4	73	12-Feb	10:00	22	22	9.45	58.4	3.86
	75		12:00	24	22	9.45	58.4	3.86
5	95	13-Feb	8:00	44	21.3	9.64	59.5	3.93
8	168.00	16-Feb	9:00	0	24.5	8.67	53.6	3.54
	171.00	(feed)	12:00	3	24.5	8.67	53.6	3.54
9	191.00	17-Feb	8:00	20	24.5	8.5	52.8	3.49
	197.50		14:30	26.5	24.5	8.5	52.7	3.48
11	216.00	19-Feb	9:00	45	22.6	8.3	52.5	3.47
	221.00	(feed)	14:00	0	23.2	8.3	52.5	3.47
12	242.00	20-Feb	11:00	21	23.2	8.14	52.3	3.46
15	264.00	23-Feb	9:00	43	25	8.3	51.8	3.43
	267.50		12:30	46.5	25	8.3	51.8	3.43
17	287.50	**25-Feb**	8:30	0	24.2	7.96	51.4	3.40
	288.50	(feed)	9:30	1	24.2	7.96	51.4	3.40
	290.00		11:00	2.5	24.2	7.96	51.4	3.40
	292.50		13:30	5	24.2	7.96	51.4	3.40

	294.00		15:00	7	24.2	7.96	51.4	3.40
	296.00		17:00	9	24.2	7.96	51.4	3.40
18	311.50	26-Feb	8:30	24.5	21.6	8.04	42.5	2.81
	312.50		9:30	25.5	21.6	8.08	42.5	2.81
	313.50		10:30	26.5	21.5	8.09	43.4	2.87
	314.50		11:30	27.5	21.5	8.09	42.6	2.82
	316.50		13:30	29.5	22.2	8.09	42.6	2.82
19	**337.00**	**27-Feb**	**10:00**	50	24.1	8.08	42.7	2.82
22	361.00	2-Mar	10:00	0	**23.8**	**7.59**	**46.4**	3.07
24	412.25	4-Mar	13:15	0hrs	21.6	8.13	61.9	4.09
	413.25		14:15	1hrs	21.6	8.14	61.9	4.09
	414.25		15:15	2hrs	21.6	8.18	61.6	4.07
	415.25		16:15	3hrs	21.5	8.14	62.1	4.11
	416.25		17:15	4rs	22.5	8.14	62.2	4.11
	417.25		18:15	5hrs	22.1	8.14	62.2	4.11
	418.25		19:15	6hrs	22	8.16	61.8	4.09
25	434	5-Mar	11:00	31hrs	23.7	8.2	51.7	3.42
	435		12:00	32hrs	23.1	8.31	51.7	3.42
	436		13:00	33hrs	23.8	8.41	51.6	3.41
	437		14:00	34hrs	**23.2**	**8.44**	**51.2**	3.39
	438		15:00	35hrs	23.2	8.46	51.1	3.38
	439		16:00	36hrs	22.7	8.48	51	3.37
26	456	6-Mar	9:00	42hrs	24	9.01	50.2	3.32
	457		10:00	43hrs	22.5	8.95	50	3.31

	458		11:00	44hrs	22.5	8.94	49.9	3.30
	459		12:00	45hrs	23.1	8.9	50	3.31
	460		13:00	46hrs	23.1	8.9	50	3.31
29	528	9-Mar	9:00	0	24	8.28	48.8	3.18
	529		10:00	1	23.9	8.29	48.9	3.19
	530		11:00	2	23.8	8.33	48.3	3.14
	531		12:00	3	23.6	8.3	48.2	3.13
	532		13:00	4	23.6	8.36	48.1	3.12
	533		14:00	5	23.5	8.44	47.4	3.07
	534		15:00	6	23.2	8.46	47.2	3.06
	535		16:00	7	23	8.46	47	3.04
	536		17:00	8	23	8.52	47	3.04
	537		18:00	9	23.2	8.48	46.9	3.04
	538		19:00	10	**23**	**8.48**	**46.7**	**3.03**
	539		20:00	11	23	8.52	46.6	3.02
	540		21:00	12	23.1	8.57	46.6	3.02
30	555	10-Mar	12:00	27	24.1	8.53	46.1	2.98
	556		13:00	28	24.1	8.53	46.1	2.98
	557		14:00	29	24.1	8.57	46	2.98
31	576	11-Mar	9:00	48	23.6	8.59	45.2	2.92
	577		10:00	49	23.6	8.78	45.1	2.91
	578		11:00	50	23.7	8.79	45.1	2.91
38	720	18-Mar	9:00	0	24.1	9.09	45.2	2.92
	721		10:00	1	13.7	7.8	48.7	3.11

	722		11:00	2	14	8.18	53.7	3.5
	723		12:00	3	18.7	8.18	54	3.53
	724		13:00	4	18.9	8.19	54	3.53
39	743	19-Mar	8:00	15	23.9	8.53	60.2	4.04
	744		9:00	16	23	8.44	60.2	4.04
	747		12:00	19	22.5	8.44	59.2	3.95
	750		15:00	21	22.3	8.52	61.1	4.09
40	768	20-Mar	9:00	39	22.4	8.53	61.2	4.1
	773.5		14:30	45	**22.5**	**8.54**	**61.4**	**4.11**

Table 7 : Data from digestor without add salinity

APPENDIX 8 :

t(h)			Paper	volume	Paper+SS	Paper+ISS	TSS	VSS	ISS	VSS/SS
							(g)	(g)	(g)	
1	39853		0.1566	0.011	0.2192	0.2058	5690.91	1218.18	4472.73	0.21406
	"0 hours"		0.1561	0.011	0.2196	0.2054	5772.73	1290.91	4481.82	0.22362
2	39854		0.1548	0.01	0.2177	0.2001	6290	1760	4530	0.27981
	"24 hours"		0.1555	0.0105	0.2202	0.2094	6161.9	1028.57	5133.33	0.16692
3	39855		0.1561	0.0105	0.2269	0.2091	6742.86	1695.24	5047.62	0.25141
	"48 hours"		0.1558	0.0125	0.2119	0.1976	4488	1144	3344	0.2549
4	39856		0.1556	0.011	0.207	0.1943	4672.73	1154.55	3518.18	0.24708
	" 52 hours"		0.1563	0.0105	0.2023	0.1907	4380.95	1104.76	3276.19	0.25217
5	39857		0.1549	0.0115	0.208	0.1926	4617.39	1339.13	3278.26	0.29002
	" 76 hours"		0.1559	0.012	0.2119	0.1977	4666.67	1183.33	3483.33	0.25357
8	39860	0.375	0.1557	0.015	0.2167	0.2053	4066.67	760	3306.67	0.18689
	(feed)		0.1563	0.01	0.2295	0.2174	7320	1210	6110	0.1653
9	39861	0.33333	0.1552	0.01	0.2187	0.2081	6350	1060	5290	0.16693
			0.156	0.012	0.2099	0.1966	4491.67	1108.33	3383.33	0.24675
11	39863	0.375	0.1545	0.013	0.2091	0.2022	4200	530.769	3669.23	0.12637
	(feed)		0.1563	0.01	0.2165	0.2091	6020	740	5280	0.12292
	39864	0.45833	0.1549	0.01	0.1952	0.1855	4030	970	3060	0.24069
			0.1552	0.011	0.1902	0.1778	3181.82	1127.27	2054.55	0.35429
12	39867	0.375	0.1565	0.011	0.184	0.1747	2500	845.455	1654.55	0.33818
			0.1563	0.01	0.1817	0.1743	2540	740	1800	0.29134
15	39869	0.35417	0.1566	0.01	0.1711	0.1651	1450	600	850	0.41379
	(feed)		0.1556	0.012	0.1953	0.1839	3308.33	950	2358.33	0.28715
17	39870	0.35417	0.1558	0.012	0.1815	0.1731	2141.67	700	1441.67	0.32685
			0.1554	0.01	0.1819	0.1726	2650	930	1720	0.35094
		0.47917	0.1556	0.013	0.1842	0.1711	2200	1007.69	1192.31	0.45804
			0.1565	0.011	0.1947	0.1843	3472.73	945.455	2527.27	0.27225
		0.5625	0.1558	0.01	0.1812	0.1743	2540	690	1850	0.27165
			0.1554	0.011	0.2086	0.1961	4836.36	1136.36	3700	0.23496
	39871	0.41667	0.1545	0.012	0.1785	0.1711	2000	616.667	1383.33	0.30833
			0.1559	0.0105	0.1744	0.1677	1761.9	638.095	1123.81	0.36216

t(h)			Paper	volume	Paper+SS	Paper+ISS	TSS	VSS	ISS	VSS/SS
							mg/l	mg/l	mg/l	
19	39874	0.41667	0.1549	0.011	0.191	0.1774	3281.82	1236.36	2045.45	0.37673
			0.1556	0.0105	0.1965	0.1824	3895.24	1342.86	2552.38	0.34474
22	39876	0.55208	0.156	0.0105	0.1962	0.1832	3828.57	1238.1	2590.48	0.32338
			0.1554	0.01	0.1841	0.174	2870	1010	1860	0.35192
		0.67708	0.1554	0.011	0.2201	0.2118	5881.82	754.545	5127.27	0.12828
			0.156	0.012	0.238	0.22	6833.33	1500	5333.33	0.21951
		0.76042	0.157	0.011	0.1925	0.1811	3227.27	1036.36	2190.91	0.32113
			0.159	0.01	0.1866	0.1767	2760	990	1770	0.3587
24	39877	0.45833	0.1539	0.014	0.2034	0.1894	3535.71	1000	2535.71	0.28283
			0.1547	0.01	0.2931	0.2706	13840	2250	11590	0.16257
		0.58333	0.1568	0.0105	0.2143	0.1929	5476.19	2038.1	3438.1	0.37217
			0.1562	0.011	0.2179	0.2173	5609.09	54.5455	5554.55	0.00972
		0.66667	0.1557	0.014	0.2028	0.19075	3364.29	860.714	2503.57	0.25584
			0.155	0.01	0.2041	0.19071	4910	1339	3571	0.27271
26	39878	0.375	0.154	0.013	0.2724	0.25	9107.69	1723.08	7384.62	0.18919
			0.1539	0.0125	0.3455	0.3172	15328	2264	13064	0.1477
		0.5	0.155	0.0115	0.2413	0.1966	7504.35	3886.96	3617.39	0.51796
			0.1544	0.013	0.2691	0.1884	8823.08	6207.69	2615.38	0.70357
29	39881	0.375	0.1538	0.0135	0.2555	0.2343	7533.33	1570.37	5962.96	0.20846
			0.1548	0.013	0.2531	0.2374	7561.54	1207.69	6353.85	0.15972
		0.5	0.155	0.01	0.2167	0.2024	6170	1430	4740	0.23177
			0.1549	0.01	0.2494	0.2301	9450	1930	7520	0.20423
		0.625	0.1547	0.0165	0.2212	0.2093	4030.3	721.212	3309.09	0.17895
			0.1545	0.013	0.2331	0.2256	6046.15	576.923	5469.23	0.09542
		0.75	0.1564	0.011	0.2594	0.2406	9363.64	1709.09	7654.55	0.18252
			0.1553	0.0105	0.2428	0.2208	8333.33	2095.24	6238.1	0.25143
	39882	0.5	0.1553	0.012	0.2254	0.2054	5841.67	1666.67	4175	0.28531
			0.1542	0.0115	0.2396	0.2297	7426.09	860.87	6565.22	0.11593
30	39883	0.41667	0.156	0.01	0.2208	0.2142	6480	660	5820	0.10185
			0.1544	0.013	0.2377	0.2102	6407.69	2115.38	4292.31	0.33013
31	39890	0.375	0.1543	0.01	0.1988	0.1902	4450	860	3590	0.19326
			0.1555	0.011	0.196	0.1898	3681.82	563.636	3118.18	0.15309

t(h)			Paper	volume	Paper+SS	Paper+ISS	TSS	VSS	ISS	VSS/SS
							mg/l	mg/l	mg/l	
38		0.5	0.154	0.013	0.2098	0.1977	4292.31	930.769	3361.54	0.21685
			0.1564	0.01	0.2128	0.2054	5640	740	4900	0.13121
	39891	0.33333	0.1563	0.011	0.2166	0.2022	5481.82	1309.09	4172.73	0.23881
			0.1566	0.012	0.2145	0.2009	4825	1133.33	3691.67	0.23489
39		0.5	0.154	0.01	0.2198	0.2043	6580	1550	5030	0.23556
			0.1556	0.012	0.2201	0.2108	5375	775	4600	0.14419
	39892	0.375	0.155	0.01	0.2176	0.2012	6260	1640	4620	0.26198
			0.1567	0.011	0.2283	0.2105	6509.09	1618.18	4890.91	0.2486
40		0.60417	0.1544	0.01	0.2305	0.2209	7610	960	6650	0.12615
			0.1552	0.012	0.2296	0.211	6200	1550	4650	0.25

Table 8: Data of VSS with salt adduction

t(h)			Paper	volume	Paper+SS	Paper+ISS	TSS	VSS	ISS	VSS/SS
							mg/l	mg/l	mg/l	
1	39853		0.1566	0.011	0.2192	0.2058	5690.909	1218.182	4472.727	0.214058
	"0 hours"		0.1561	0.011	0.2196	0.2054	5772.727	1290.909	4481.818	0.223622
2	39854		0.1548	0.01	0.2177	0.2001	6290	1760	4530	0.279809
	"24 hours"		0.1555	0.0105	0.2202	0.2194	6161.905	76.19048	6085.714	0.012365
3	39855		0.1561	0.0105	0.2269	0.2091	6742.857	1695.238	5047.619	0.251412
	"48 hours"		0.1558	0.0125	0.2119	0.1976	4488	1144	3344	0.254902
4	39856		0.1556	0.011	0.207	0.1943	4672.727	1154.545	3518.182	0.247082
	" 52 hours"		0.1563	0.0105	0.2023	0.1907	4380.952	1104.762	3276.19	0.252174
5	39857		0.1549	0.0115	0.208	0.1926	4617.391	1339.13	3278.261	0.290019
	" 76 hours"		0.1559	0.012	0.2119	0.1977	4666.667	1183.333	3483.333	0.253571
8	39860	0.375	0.1572	0.01	0.1905	0.1768	3330	1370	1960	0.411411
	(feed)		0.1562	0.01	0.1875	0.1739	3130	1360	1770	0.434505
9	39861	0.333333	0.1562	0.01	0.1977	0.1881	4150	960	3190	0.231325
			0.1544	0.012	0.1904	0.1806	3000	816.6667	2183.333	0.272222
11	39863	0.375	0.1545	0.013	0.1899	0.1813	2723.077	661.5385	2061.538	0.242938
	(feed)		0.1563	0.01	0.1965	0.1891	4020	740	3280	0.18408
		0.583333	0.155	0.012	0.1972	0.1901	3516.667	591.6667	2925	0.168246
			0.1566	0.01	0.1919	0.1865	3530	540	2990	0.152975
12	39864	0.458333	0.1548	0.01	0.1741	0.1668	1930	730	1200	0.378238
			0.157	0.011	0.1804	0.1713	2127.273	827.2727	1300	0.388889
15	39867	0.375	0.1552	0.013	0.1781	0.1663	1761.538	907.6923	853.8462	0.515284
			0.1577	0.011	0.1745	0.1681	1527.273	581.8182	945.4545	0.380952

t(h)			Paper	volume	Paper+SS	Paper+ISS	TSS	VSS	ISS	VSS/SS
							mg/l	mg/l	mg/l	
17	39869	0.354167	0.1574	0.0115	0.1818	0.1736	2121.739	713.0435	1408.696	0.336066
	(feed)		0.1579	0.0105	0.1827	0.1742	2361.905	809.5238	1552.381	0.342742
	39870	0.354167	0.1552	0.011	0.209	0.1972	4890.909	1072.727	3818.182	0.219331
			0.1571	0.0105	0.1806	0.17	2238.095	1009.524	1228.571	0.451064
		0.479167	0.1559	0.01	0.1798	0.1703	2390	950	1440	0.39749
			0.1578	0.0105	0.2112	0.199	5085.714	1161.905	3923.81	0.228464
		0.5625	0.1568	0.0145	0.1824	0.1721	1765.517	710.3448	1055.172	0.402344
			0.156	0.0105	0.1865	0.1753	2904.762	1066.667	1838.095	0.367213
19	39871	0.416667	0.1557	0.0125	0.1756	0.1687	1592	552	1040	0.346734
			0.1562	0.01	0.1709	0.1653	1470	560	910	0.380952
22	39874	0.416667	0.156	0.01	0.1927	0.1814	3670	1130	2540	0.307902
			0.1553	0.012	0.1867	0.1724	2616.667	1191.667	1425	0.455414
24	39876	0.552083	0.1563	0.01	0.2152	0.2018	5890	1340	4550	0.227504
			0.1549	0.011	0.2166	0.2029	5609.091	1245.455	4363.636	0.222042
		0.677083	0.1565	0.0115	0.1965	0.183	3478.261	1173.913	2304.348	0.3375
			0.1559	0.0115	0.2308	0.2062	6513.043	2139.13	4373.913	0.328438
		0.760417	0.1557	0.015	0.2412	0.2225	5700	1246.667	4453.333	0.218713
			0.1565	0.014	0.2206	0.2071	4578.571	964.2857	3614.286	0.210608
25	39877	0.458333	0.1572	0.01	0.2092	0.1945	5200	1470	3730	0.282692
			0.1562	0.01	0.2041	0.1898	4790	1430	3360	0.298539
		0.583333	0.1551	0.013	0.2173	0.2018	4784.615	1192.308	3592.308	0.249196
			0.1556	0.013	0.2306	0.2179	5769.231	976.9231	4792.308	0.169333
		0.666667	0.1554	0.01	0.2053	0.1909	4990	1440	3550	0.288577
			0.1547	0.011	0.1999	0.1909	4109.091	818.1818	3290.909	0.199115

t(h)			Paper	volume	Paper+SS	Paper+ISS	TSS	VSS	ISS	VSS/SS
							mg/l	mg/l	mg/l	
26	39878	0.375	0.1566	0.012	0.2342	0.2196	6466.667	1216.667	5250	0.188144
			0.1538	0.011	0.2315	0.2159	7063.636	1418.182	5645.455	0.200772
		0.5	0.1534	0.01	0.2322	0.1816	7880	5060	2820	0.642132
			0.1529	0.014	0.2291	0.1849	5442.857	3157.143	2285.714	0.580052
29	39881	0.375	0.1542	0.115	0.2373	0.2194	722.6087	155.6522	566.9565	0.215403
			0.1537	0.019	0.2768	0.2529	6478.947	1257.895	5221.053	0.194151
		0.5	0.1545	0.01	0.2444	0.2256	8990	1880	7110	0.209121
			0.1552	0.01	0.2075	0.1952	5230	1230	4000	0.235182
		0.625	0.1547	0.013	0.1952	0.1808	3115.385	1107.692	2007.692	0.355556
			0.1551	0.01	0.2435	0.1901	8840	5340	3500	0.604072
		0.75	0.1557	0.011	0.2488	0.2276	8463.636	1927.273	6536.364	0.227712
			0.155	0.0105	0.2428	0.2325	8361.905	980.9524	7380.952	0.117312
30	39882	0.5	0.1544	0.01	0.2254	0.2054	7100	2000	5100	0.28169
			0.1567	0.01	0.2396	0.2297	8290	990	7300	0.119421
31	39883	0.416667	0.1554	0.012	0.2208	0.2142	5450	550	4900	0.100917
			0.1554	0.0105	0.2377	0.2102	7838.095	2619.048	5219.048	0.334143
38	39890	0.375	0.155	0.01	0.1888	0.1822	3380	660	2720	0.195266
			0.1561	0.011	0.1931	0.1865	3363.636	600	2763.636	0.178378
		0.5	0.154	0.013	0.1989	0.1879	3453.846	846.1538	2607.692	0.244989
			0.1563	0.01	0.2034	0.1954	4710	800	3910	0.169851
39	39891	0.333333	0.156	0.011	0.2056	0.1999	4509.091	518.1818	3990.909	0.114919
			0.1576	0.012	0.1989	0.1905	3441.667	700	2741.667	0.20339
		0.5	0.154	0.01	0.2044	0.1943	5040	1010	4030	0.200397
			0.155	0.012	0.2019	0.1908	3908.333	925	2983.333	0.236674
t(h)			Paper	volume	Paper+SS	Paper+ISS	TSS	VSS	ISS	VSS/SS
							mg/l	mg/l	mg/l	
40	39892	0.375	0.1542	0.01	0.2079	0.1999	5370	800	4570	0.148976
			0.156	0.011	0.2085	0.2001	4772.727	763.6364	4009.091	0.16
		0.604167	0.1554	0.01	0.2005	0.1935	4510	700	3810	0.155211
			0.1542	0.012	0.2196	0.2077	5450	991.6667	4458.333	0.181957

Table 9: Data of VSS without salt adduction