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Synthetic urban wastewater treatment by an activated sludge reactor: Evolution of bacterial biomass and purifying efficiency

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Abstract

The present study aims to assess the evolution of biomass growth and the performances of an activated sludge pilot plant treating a synthetic urban wastewater. The activated sludge pilot plant used in this experiment reproduces the main principles of biological treatment using activated sludge process. The samples were collected every working day from the inlet, the outlet and in the bioreactor. The parameters monitored according to standards methods are: biomass growth, total suspended solids (TSS), volatile suspended solids (VSS), organic load (BOD_5 and COD) and nutrients (nitrogen and phosphorus). Results showed a successful growth of the biomass that reached 1.62±0.88 g_{TSS}/L and 1.30±0.77 g_{VSS}/L. The activated sludge pilot plant performances in term of TSS, Turbidity, BOD_5 and COD_T average removal efficiencies exceeded the expected performances and reached 90.6%, 91.5%, 83.6% and 90.8%, respectively. Although, the final effluent measured parameters concentrations were bellow and or close to design criteria of the pilot plant and the Moroccan applied discharge standards. Overall, the results obtained from this experimental study suggest that the activated sludge pilot plant has performed adequately under conditions equal to or less than its original design capacity and complained with the Moroccan applied discharge standards.

1. Introduction

Currently, environmental pollution by organic compounds is a worldwide issue and has posed serious threaten towards environmental and human health. Several methods including flotation [1]; flocculation [2]; reverse osmosis [3]; electrocoagulation [4]; electrochemical oxidation [5]; adsorption [6] and biological methods [7,8] were exploited to achieve the efficient removal of organic pollutants.

Among the biological available technologies, activated sludge biological process has been employed to treat a wide variety of wastewater and over 90% of the municipal wastewater treatment plants use it as the core part of the treatment process. In which the biological degradation of both soluble organic and inorganic components and particulate matter carried out by microbial flocs, which are traditionally separated from the liquid stream through gravity sedimentation. The most common suspended growth process used for municipal wastewater treatment is the activated sludge process. The process uses a mixed culture of microorganisms that degrade the wastewater aerobically producing carbon dioxide, water and new biomass. In activated sludge process, wastewater containing organic matter is aerated in an aeration tank in which microorganisms metabolize the suspended and soluble organic matter. Part of organic matter is synthesized into new cells and part is oxidized to carbon dioxide and water to derive energy.

In the bioreactor of an activated sludge wastewater treatment plant (WWTP), the new cells formed in the reaction are removed from the liquid stream in the form of a flocculent sludge in settling tanks. A part of this settled biomass, described as activated sludge is returned to the aeration tank and the remaining forms waste or excess sludge:

$$\text{Organic matter} + \text{Microorganisms (Biomass)} + O_2 \rightarrow CO_2 + H_2O + \text{Biomass} \quad \text{Eq.1}$$

Before discharging wastewater into environment, removing nitrogen and phosphate is usually obligatory, even though in many cases it is not performed and leads to major contamination on a worldwide level. These nutrients are directly responsible for eutrophication (extraordinary growth of algae as a result of excess nutrients in water bodies) of rivers, lakes, and seas worldwide [9]. Consequently, disposal of wastewaters produces a constant threat to dwindling fresh water on a global scale [10]. However, nitrogen and phosphorus compounds can be removed from wastewater by a variety of physicochemical and biological processes.

The current work aims to investigate and to evaluate process loading and the performances of an activated sludge pilot plant during a treatment cycle, through the assessment of temporal variations of the main physicochemical parameters. Indeed, biomass growth, Total suspended solids TSS, organic load (5 day biological oxygen demand BOD_5 and chemical oxygen demand COD) and nutrients (nitrogen and phosphorus) were evaluated and assessed over the time.

2. Material and Methods

2.1. *Description of the activated sludge pilot plant*

The activated sludge pilot plant used in this experiment is presented in the Figure 1.

The pilot plant reproduces the operation of the activated sludge process full-scale plant. It consists of an anoxic tank (18 L), an aeration tank (60 L), a settling tank (30 L), a substrate vat (100 L), a circulating pump and an aeration system (Figure.1). This installation aims to highlight the main principles of biological treatment using activated sludge process.

The pilot is equipped with various analytical instruments, including probes for oxygen, pH and redox potential. All equipment were connected to a central programmable logic controller (PLC) linked to a supervisory control and data acquisition (SCADA): PLC SOFREL, which is a single program, manages the automation and supervision providing a direct access and data storage.

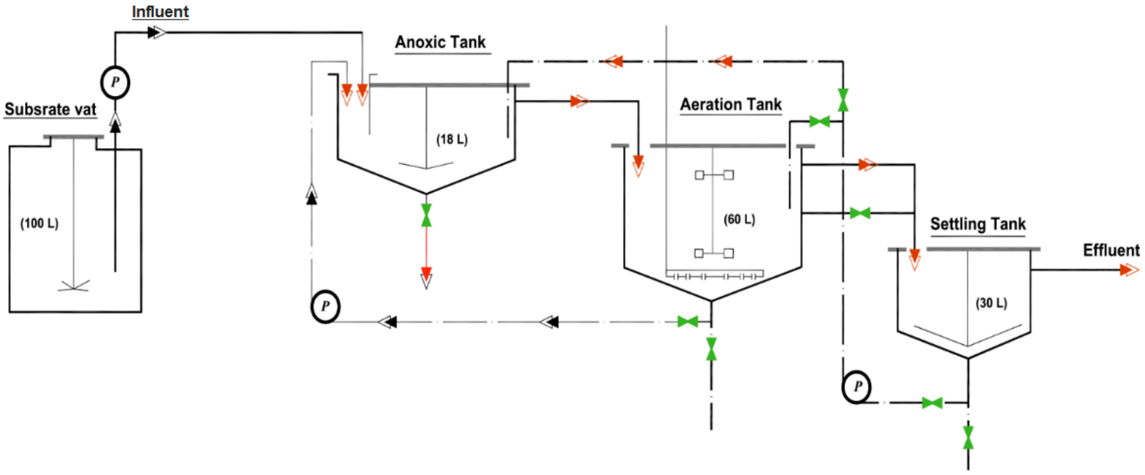


Figure 1: Schematic diagram of the pilot-scale activated sludge plant **[7]**

2.2 *Operation of the pilot system*

2.2.1. *Activated sludge source*

For the start-up of the pilot plant of activated sludge, the aeration tank and the settling tank were filled with activated sludge and wastewater collected from the aeration tank and settling tank of Marrakech wastewater treatment plant activated sludge. The main physicochemical characteristics of the activated sludge and wastewater used in this experiment are presented in Table 1.

- The aeration tank of the pilot plant is filled with 60 L of the biomass (3.44 g/L) picked up from the aeration tank of the Marrakech wastewater treatment plant.
- The settling tank (30 L) is filled with wastewater collected from the secondary sedimentation tank of the Marrakech wastewater treatment plant.

2.2.2. *Synthetic urban wastewater preparation*

The synthetic wastewater used in this experimental study is obtained by mixing several compounds. It is mainly formed of a source of carbon (saccharose; $C_{12}H_{22}O_{11}$), a source of amino acids (Viandox), a source of sodium chloride (NH_4Cl) and a source of phosphate (H_3PO_4). Its composition is similar to urban wastewater in order to pefrom reproducible experiments and is frequently used in this kind of investigation as tested in several research studies [11-13].

Table 1: Characteristics of wastewater collected from the aeration and secondary sedimentation tanks from Marrakech wastewater treatment plant

	Aeration tank	**Settling tank**
TSS (g/L)	3.44	-
Turbidity (FTU)	2833	1.79
COD_T (mg/L)	1039	70
BOD_5 (mg/L)	620	-
PO_4^{3-}(mg/L)	4.20	2.77
NO_2^- (mg/L)	0.14	0.24
TP (mg/L)	7.75	3.96
TKN (mg/L)	28	16.6
NO_3^- (mg/L)	0.61	1.40

In this study, we used Maggi beef instead of Viandox that has the same role. The synthetic wastewater is prepared as a concentred solution from a predetermined portion of different compounds with a COD of about 500 mg/L as showed in Table 2.

Table 2: Synthetic urban wastewater composition

Viandox (cubes of Maggi)	**$C_{12}H_{22}O_{11}$**	**NH_4Cl**	**H_3PO_4**	**H_2O**
18 cubes	8.75 g	7.38 g	1.1 mL	Water non chorea q.s 100 L.

2.2.3. Operation of the pilot system

In order to investigate the biomass evolution and the system performances in term of TSS, COD, BOD_5, TKN and TP removal efficiencies, the pilot plant of activated sludge is operated for 20 days (24h/24h) under the following experimental conditions:

- The hydraulic retention time (HRT) in the bioreactor was about 10 h.
- The ambient temperature was approximately 30 °C.
- In the aeration tank, oxygen was provided by an air pump regulated to maintain residual dissolved oxygen concentration between 2 mg/L and 3 mg/L.
- Recycling rate/flow rate Ratio was set at 1 to ensure the presence of a significant amount of biomass in the reactor.

2.3 *Analytical methods*

2.3.1 *Physicochemical parameters*

The pilot plant performances have been investigated during a period cycle of 20 days (475 hours). Samples were collected every working day and analysed in laboratory. Total suspended solids (**TSS**), Turbidity, volatile suspended solids (**VSS**), chemical oxygen demand (**COD**), 5-day biological oxygen demand (**BOD_5**), total kjeldhal nitrogen (**TKN**), nitrate (**NO_3^-**), nitrite (**NO_2^-**), orthophosphate (**PO_4^{3-}**) and total Phosphorus (**TP**) were monitored according to standards methods [14-16].

2.3.2 *Instantaneous measurements*

The instantaneous measurements were performed directly in the pilot plant via automat system. Therefore, pH, redox potential, temperature, and dissolved oxygen in the pilot plant were recorded every 5 minutes continuously.

3. Results and Discussion

3.1. *Biomass growth in the bioreactor*

The most common parameter used for quantifying activated sludge in the bioreactor, in a WWTP, is the content of suspended solids, expressed as total suspended solids (TSS) or volatile suspended solids (VSS). However, VSS do not coincide with the effective bacterial biomass in activated sludge because VSS also include endogenous biomass (the residue produced by bacterial death and lyses) and organic non-biotic particulate matter fed into the plant with the wastewater influent. However, knowledge of the actual amount of bacterial biomass in an activated sludge pilot system is a key parameter for understanding the processes evolution, kinetics and dynamics of substrate removal.

The biomass evolution in the bioreactor in term of TSS and VSS is presented in the Figure 2. Overall this experiment, the TSS and VSS average concentrations were 1.62±0.88 g/L and 1.30±0.77 g/L, respectively. The average of the ratio VSS/TSS was about 77±0.1%. The biomass evolution in the aeration tank of the investigated activated sludge pilot plant (ASPP) is done according to the growth curve following four distinct phases:

Lag phase: activated sludge is introduced into the bioreactor of ASPP, it takes some time to adapt with the new environmental conditions. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate and therefore no increase in cell mass. The length of this phase depends directly on the previous growth condition of the biomass. However, when the microorganism growing in a rich medium is inoculated into nutritionally poor medium, the biomass will take more time to adapt with the new environment. The microorganisms will start synthesising the necessary proteins, co-enzymes and vitamins needed for their growth and hence there will be a subsequent increase in the lag phase. Similarly when microorganisms from a nutritionally poor medium are added to a nutritionally rich medium, they can easily adapt to the environment, they can start the cell division without any delay, and therefore they will have less lag phase which it may be absent. In this study the lag phase lasted in four days. It separates the time of inoculation and the moment when biomass growth becomes noticeable (Figure 2).

Exponential phase: during this phase, the microorganisms are in a rapidly growing and dividing state after their adaptation and acclimation to new environmental conditions and synthetic wastewater composition. Their metabolic activity increases and the microorganisms begin the DNA replication by binary fission at a constant rate. The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases exponentially.

As shown in Figure 2, during this phase the amount of biomass (TSS and VSS) varies linearly over time. The observed rate is equal to the rate of synthesis; the phenomenon of endogenous respiration is negligible. Oxygen demand of the cells during this phase is somewhat variable depending on the species [17].

Stationary phase: as the bacterial population continues to grow, all the nutrients in the growth medium are used up by the microorganisms for their rapid multiplication. This results in the accumulation of waste materials, toxic metabolites and inhibitory compounds such as antibiotics in the medium. This shifts the conditions of the medium such as pH and temperature, thereby creating an unfavourable environment for the bacterial growth. The reproduction rate will slow down, the cell undergoing division is equal to the number of cell death, and finally bacterium stops its division completely. The cell number is not increased and thus the growth rate is stabilised. If a cell taken from the stationary phase is introduced into a fresh medium, the cell can easily move on the exponential phase and will be able to perform its metabolic activities as usual.

Probably, the stationary phase corresponds to a disturbance of the culture medium by the exponential growth of microorganisms. The passage in slowdown phase corresponds to a decrease in the rate of biomass growth [18].

Decline phase: the depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitates the bacterium to move onto the decline phase. During this phase, the bacterium completely loses its ability to reproduce. Individual bacteria begin to die due to the unfavorable conditions and the death is rapid and at uniform rate. The number of dead cells exceeds the number of live cells. Some microorganisms which can resist to this condition can survive in the environment by producing endospores. In our case, this phase correspond to the end of the treatment cycle.

The monitoring of the biomass growth during the time of this experiment reveals the presence of an optimal amount of biomass during the experiment. These results highlight a successful adaptation and selection of a biomass able to remove pollutants effectively from the synthetic urban wastewater.

3.2. *Performances of the activated sludge pilot plant*

3.2.1 *TSS removal efficiency*

Total Suspended Solids (TSS) is a key measurement in a wastewater treatment plant in order to assess system performance and treated wastewater quality. However, the amount of TSS in the effluent affects the efficiency of the disinfection process and defines the recommended TSS value of discharged effluent.

Figure 3 shows the influent and effluent TSS and Turbidity (FTU) concentrations and their average removal efficiency during the experiment.

The input average of TSS and Turbidity were 221.43±67.35 mg/L and 199.93±55.86 mg/L, respectively. Whereas, the outputs average TSS and Turbidity were 20.71±5.5 mg/L and 17.06±4.2 mg/L, respectively. These values satisfied Moroccan applied discharge standards (TSS<30 mg/L) [19].

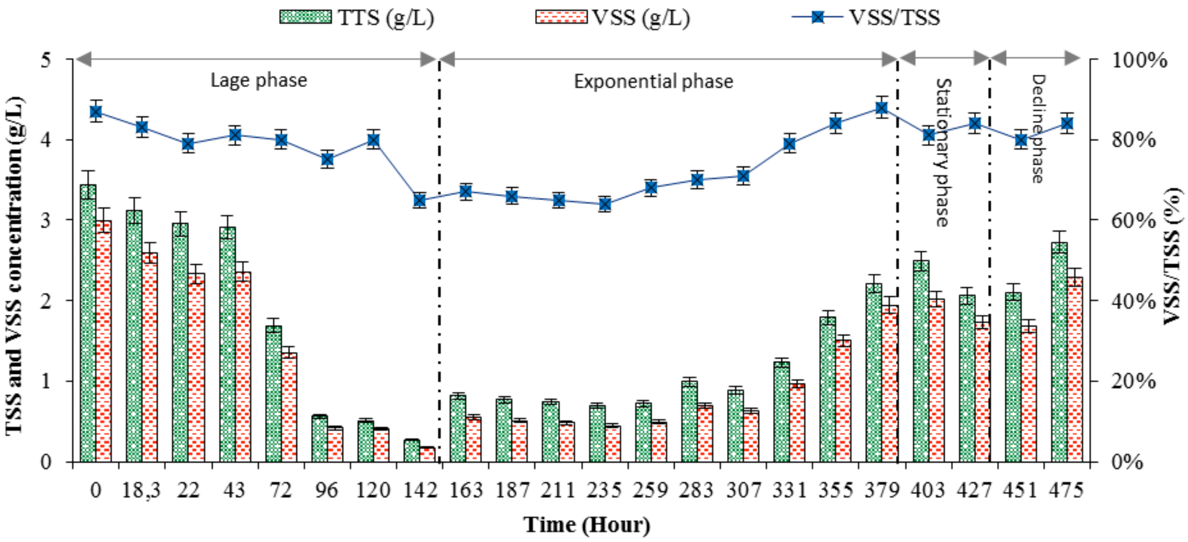
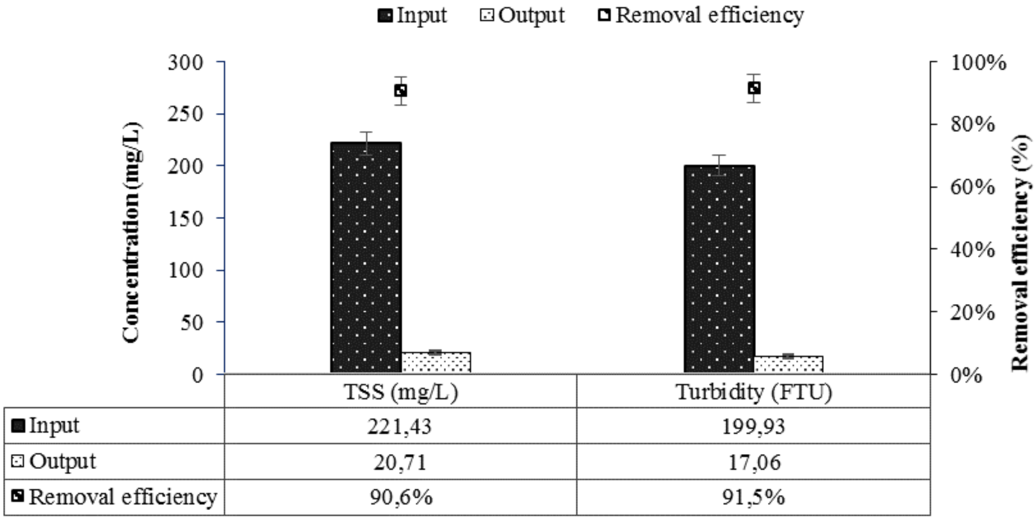


Figure 2: Biomass evolution in aeration tank of the activated sludge pilot plant over the time



	TSS (mg/L)	Turbidity (FTU)
■ Input	221,43	199,93
⊡ Output	20,71	17,06
◘ Removal efficiency	90,6%	91,5%

Figure 3: Total suspended solids and Turbidity removal efficiency during the experiment

The average removal efficiencies in term of TSS and Turbidity were about 90.6% and 91.5%. This result indicates high performance by the pilot plant during this experiment. In addition, the obtained results are close to those reported in the investigation study in full scale Marrakech activated sludge WWTP [20].

3.2.2. *Organic load removal*

a) *COD and BOD$_5$ evolution in the activated sludge pilot plant*

Wastewater treatment is mainly concerned with the biodegradation of organic carbon or BOD. This is carried out by the heterotrophic or carbonaceous bacteria in the aeration tank of the WWTP. These bacteria take up, and hence remove, organic carbon molecules from the mixed liquor, and use them for either respiration or for the growth of new biomass. However, activated sludge biological process refers to a biomass (pollution of microorganisms) able to break down organic compounds into carbon dioxide (CO_2), water (H_2O) and other inorganic compounds. In a WWTP, the COD and BOD$_5$ are used as the surrogate parameters to measure the organic matter available for the microorganisms in the bioreactor and to define its concentration in the influent and effluent. The BOD$_5$ test has been in use for more than a century, and consequently remains deeply entrenched in the practice and experience of biological WWTP. The COD analysis estimates the amount of organic matter in wastewater in only three to four hours, rather than the five days required by the BOD$_5$ test, and can be used as an alternative [21].

Figure 4 shows organic load evolution (BOD$_5$ and COD) in different treatment levels of the activated sludge pilot plant over the time.

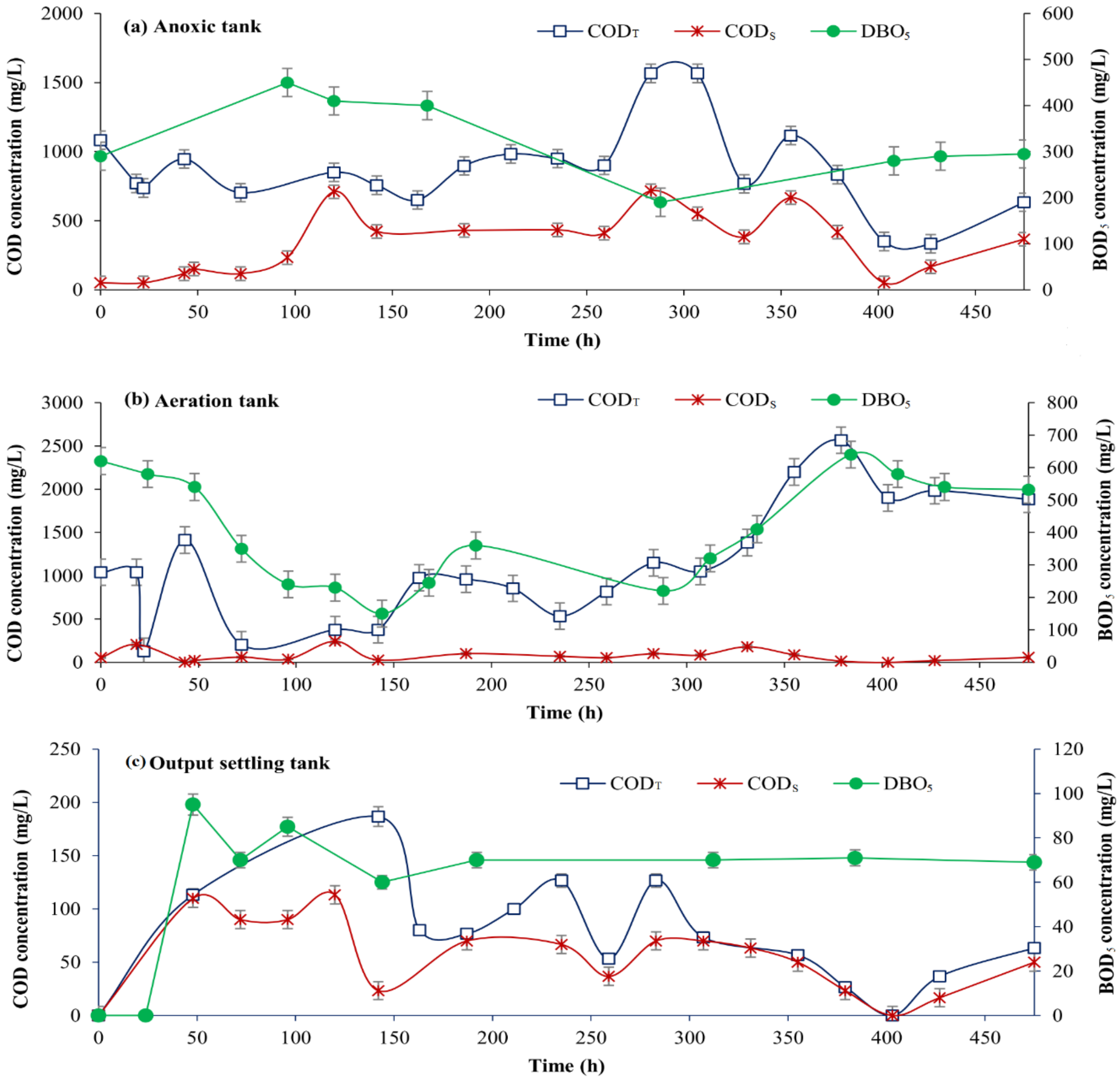


Figure 4: Organic load evolution (BOD_5 and COD) in the activated sludge pilot plant over time (a) Anoxic tank (b) Aeration tank and (c) Output settling tank

In the anoxic tank (Figure 4a): COD varies slightly during the first week since we control the composition of the injected substrate. The 5-day biological oxygen demand BOD_5, the soluble chemical oxygen demand CODs and the total chemical oxygen demand COD_T input concentrations were 325.63±70.78 mg/L, 869.58±218.27 mg/L and 338.91±217 mg/L, respectively. Beyond, COD varies proportionally to the biomass growth. In fact, the biomass was injected 7 days after the startup of the ASPP.

In the aeration tank (Figure 4b): BOD_5 and COD_T vary proportionately; the shape of the two curves follows the evolution of that of the biomass. In addition, their concentrations were 409.81±145 mg/L and 1146.90±558.24 mg/L, respectively. CODs concentration was about 74.55±22 mg/L indicating a good biomass activity in the bioreactor of ASPP.

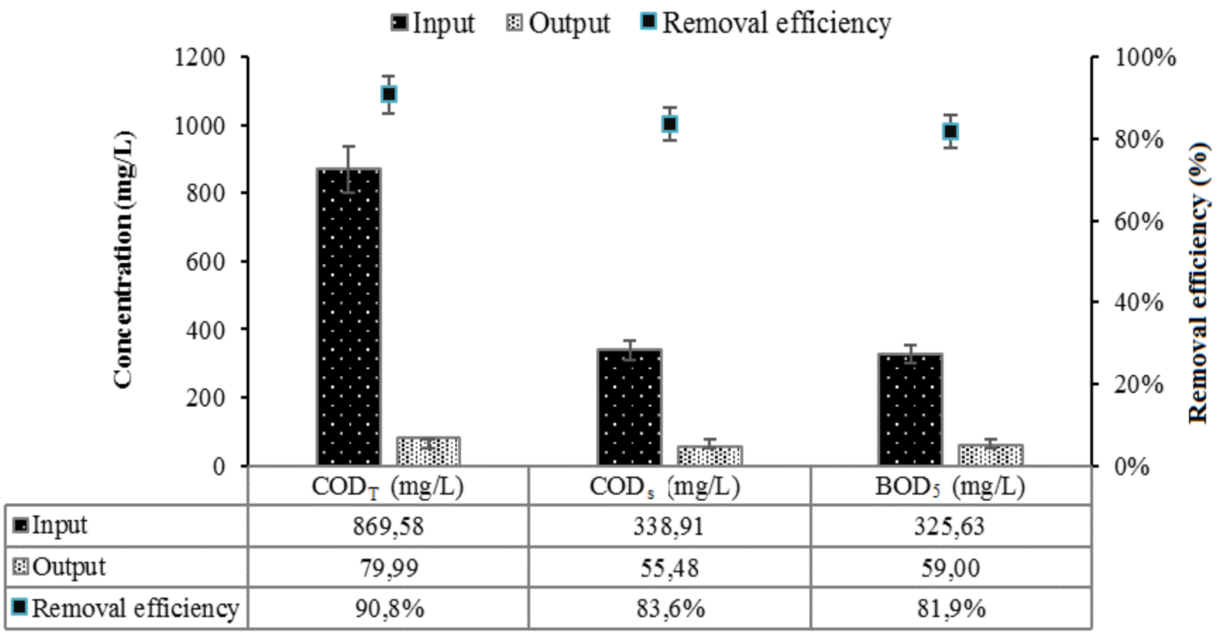
In the output of settling tank (Figure 4c): the output BOD_5 concentration was about 59.00±23.60 mg/L, this value exceeded the Moroccan applied discharge standards (BOD_5<40 mg/L) [19]. Moreover, the COD_T average concentration was 80.00±36.20 mg/L, which is below the Moroccan applied discharge standards (COD < 120 mg/L) [19].

Our results are close to those reported in Marrakech full scale activated sludge wastewater treatment plant [20].

b) COD and BOD5 removal efficiency

Figure 5 reports the organic load (BOD_5 and COD) removal efficiency. BOD_5 and COD_T abatements during this experiment were around 83.6% and 90.8 % which indicate an efficient treatment of organic matter by the activated sludge pilot plant. The obtained results were close to the results reported in full scale activated sludge wastewater treatment plant treating urban wastewater [20]. These results confirm that the activated sludge pilot plant performed well with respect to the organic matter removal efficiency within the investigated period and complied with Moroccan applied discharge standards [19].

Figure 5: Organic load (COD and BOD_5) removal efficiency



	COD_T (mg/L)	COD_s (mg/L)	BOD_5 (mg/L)
■Input	869,58	338,91	325,63
▨Output	79,99	55,48	59,00
■Removal efficiency	90,8%	83,6%	81,9%

3.2.3 *Nutrients evolution*

Nitrogen exists in many forms in wastewater including primarily ammonia as well as nitrite (NO_2^-), nitrate (NO_3^-) and organic nitrogen compounds. In order to effectively reduce nitrogen concentration in WWTP effluent via biological denitrification, other forms of nitrogen should first be converted into nitrates. The process of biological nitrification oxidizes ammonia into nitrite and then to nitrate [22], which is reduced to nitrogen gas by heterotrophic and autotrophic bacteria through nitrogen respiration [23].

In the anoxic tank (Figure 6a), concentrations of nitrates and nitrites are variable during the first ten days which correspond to the period of biomass acclimation. Beyond this period, the concentrations of nitrates and nitrites are almost negligible. These values characterize an effective denitrification in the anoxic tank. In anoxic medium, nitrate ion plays the role of an electron acceptor, as illustrated in the following equation (Eq2):

$$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O \quad \textbf{\textit{Eq.2}}$$

Indeed, CH_2O represents a hydro-carbonaceous organic substrate. The reaction is theoretically spontaneous ($\Delta G < 0$), it is performed by anaerobic microorganisms. This is denitrification, and it is realized by steps:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad \textbf{\textit{Eq. 3}}$$

In the aeration tank (Figure 6b), an increase in nitrate concentration in the treated water was observed. This increase may be explained by a reaction of nitrification of organic nitrogen in the effluent. This same observation was noted in the work of [22].

The same author stipulates that during the biological treatment of wastewater, NH_4-N (ammonia nitrogen) is converted into NO_2-N (nitrite) then into NO_3-N (nitrate) due to the oxygen injection in aeration tank.

Nitrification is a two-step process in which the ammonia is oxidized to nitrite then to nitrate **[23]**:

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + 2H^+ + 2H_2O \quad \text{Then} \quad NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \quad \textbf{\textit{Eq. 4}}$$

In the output of settling tank (Figure 6c), the TKN output concentration varied from 33.6±5.10 mg/L to 11.2±2.5 mg/L, with an average concentration of 19.47±5.23mg/L, which is above European Standards (10 to 15 mg/L) [24] but bellow Moroccan applied discharge standards (40 mg/L) [19].

3.2.4. *Phosphorus evolution*

Removing phosphate by biological means can be accomplished by two independent mechanisms: (i) direct absorption of phosphorus by suspended growing cells and plants, and (ii) enhancing the storage capacity of phosphorus as polyphosphate by the microbial biomass in activated sludge at the treatment facility. In activated sludge wastewater treatment plant, bacteria only use enough phosphorus to satisfy their basic metabolic requirements, resulting in typical removal rates of 20-40% [25, 26].

In the anoxic tank (Figure 7a), during the anaerobic phase, the release of phosphorus is not a linear function of time. Three phases can be distinguished :

- An increase of the concentration of total phosphorus in the basin through the accumulation effect during the first three days;
- Between the third and fifth days, there is a phenomenon of salting out of phosphate in the external medium by bacteria probably due to a super saturation of phosphorus in the tank. The speed of this process is independent of the carbon concentration of readily assimilable essentially volatile fatty acids;
- Beyond the fifth day, slowing salting out occurs due to the use of carbon substrates that require prior hydrolysis. Then a slow salting out, due to the maintenance of the cell. It comes the salting out secondary or endogenous.

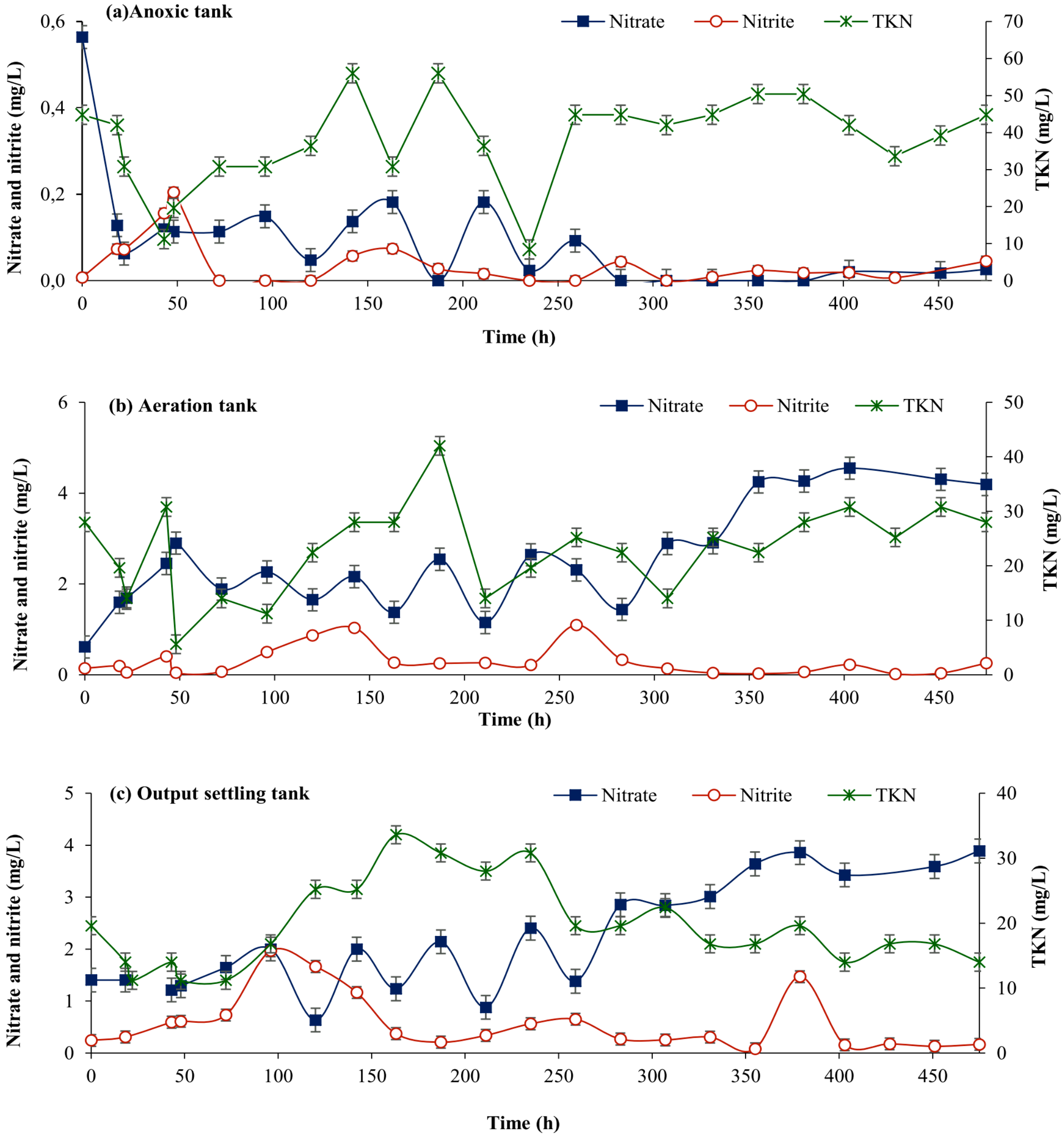


Figure 6: Nitrogen evolution (TKN, NO_2 and NO_3) over the time; (a) Anoxic tank (b) Aeration tank and (c) Output settling tank

In the aeration tank (Figure 7b), we find that the evolution of phosphorus is inversely proportional to the evolution of the biomass. Indeed, poly-β-alkanoates (PHA) and the organic matter contained in wastewater are oxidized by bacteria. Respiration produces the necessary energy for bacteria that regenerate their stocks of polyphosphates and growth [27].

In the output of settling tank (Figure 7c), the concentrations of phosphorus in term of orthophosphate and total phosphorus were 4.91±2.30 mg/L and 5.59±3.47 mg/L, respectively. The TP concentration in treated effluent is above the Moroccan applied discharge standards (2 mg/L) [19].

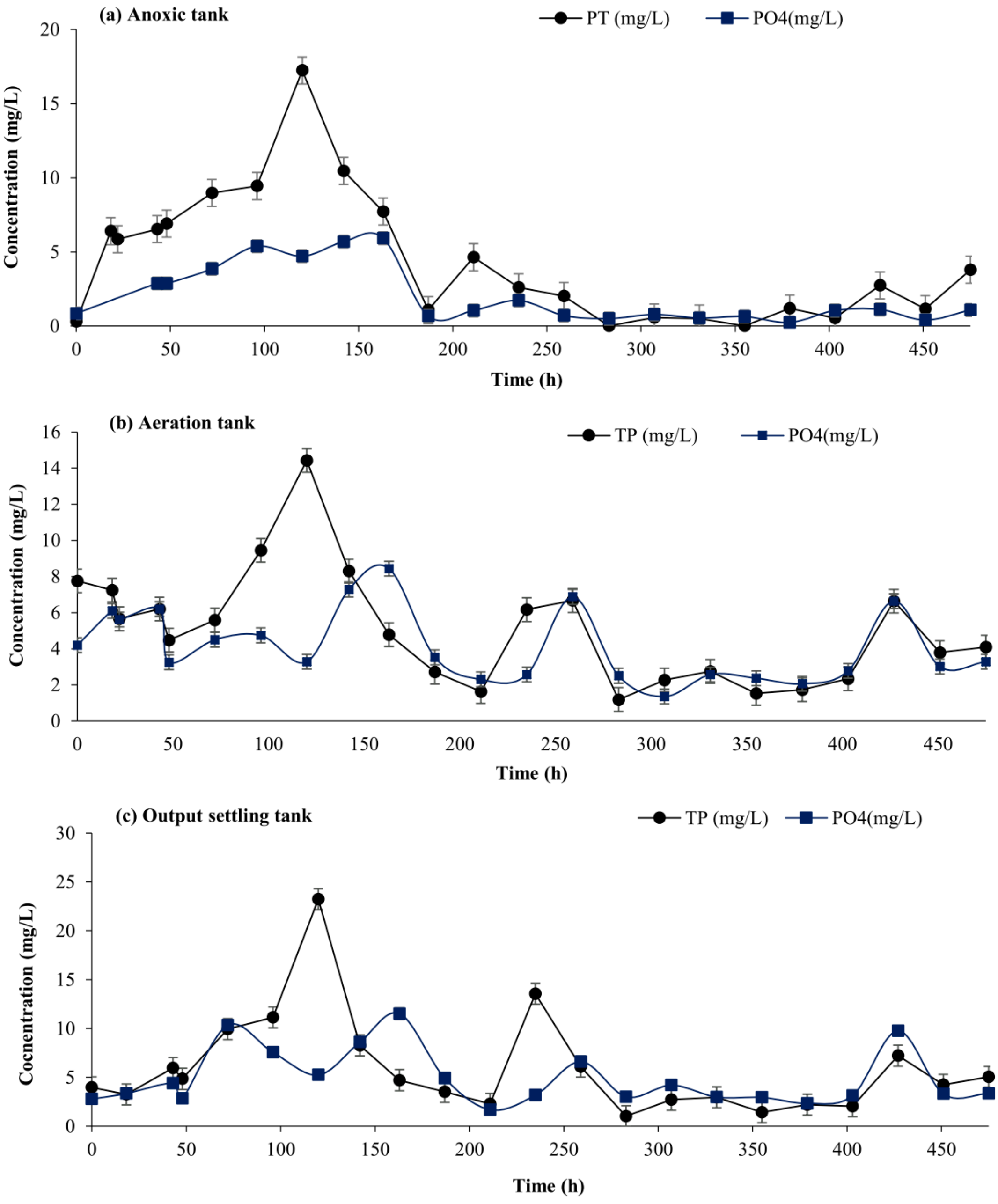


Figure 7: Phosphorus evolution (TP and PO_4) over time (a) anoxic tank (b) Aeration tank and (c) Output settling tank

3.3. *Instantaneous measurements*

Instantaneous measurement such as, pH, redox potential (Redx mV), temperature (t °C) and dissolved oxygen (DO mg/L) were performed in order to monitor the pilot plant operating parameters. Figure 8 shows the evolution of the different parameters measured instantaneously during this experiment.

The pH of the anoxic tank is relatively neutral (between 6.8 and 7.8). This pH is the optimum pH for growth of purifying bacteria.

Dissolved oxygen in the anoxic tank is almost zero (≈ 0.03 mg/L). Therefore, the redox potential of the medium is reducer (≈ -380 mV), which is optimal for denitrification process.

The optimal dissolved oxygen concentration is maintained in the aeration tank via pump system. Indeed, its concentration was between 2.31 mg/L and 2.61mg/L. This optimal concentration in the bioreactor ensures oxygen requirements necessary for the development of biomass able to degrade effectively organic matter.

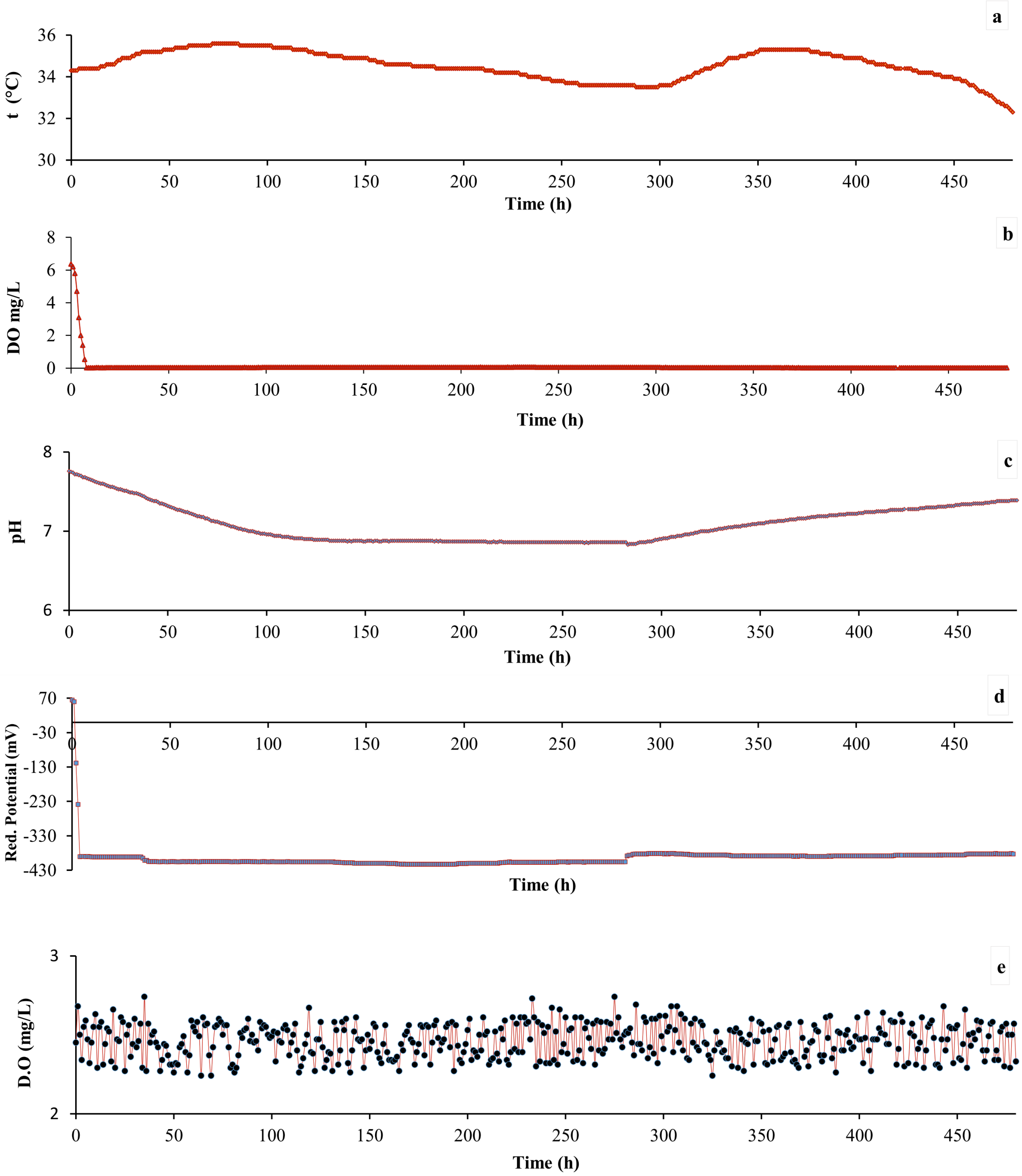


Figure 8: Evolution of measured parameters over the time
Anoxic tank: (a) Temperature; (b) Dissolved oxygen; (c) pH and (d) Redox potential
Aeration tank: (e) Dissolved oxygen

Conclusion

The obtained results in this study highlight that the activated sludge pilot plant practically performed under the experimental conditions. This was confirmed by the successful growth of the biomass in the bioreactor that reached 1.62±0.88 g_{TSS}/L and 1.30±0.77 g_{VSS}/L. The performances in term of TSS, Turbidity, BOD_5 and COD_T averages removal efficiencies exceeded the expected performances and reached 90.6%, 91.5%, 83.6% and 90.8% respectively. The output effluent measured parameters concentrations were bellow and or close to design criteria of the pilot plant and Moroccan applied discharge standards. Indeed, TSS, BOD_5, COD_T, TKN and TP output average concentrations were 20.71±5.5 mg/L, 59.00±23.60 mg/L, 80.00±36.20 mg/L, 19.47±5.23 mg/L and 5.59±3.47 mg/L respectively. These results highlight overall performances of the activated sludge pilot plant due to selection and adaptation of a biomass able to remove effectively the pollutants from synthetic urban wastewater.

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