Effects of C/N Ratio and DO concentration on Carbon and Nitrogen removals in a Hybrid Biological Reactor

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ABSTRACT

The objective of this paper was to evaluate the effects of dissolved oxygen (DO) concentration and C/N ratio on the biological removal of carbon and nitrogen using an Integrated Fixed-film Activated Sludge (IFAS) reactor for nearly half a year. The results showed that the efficiency of organic matter removal was greater than 90% regardless of the value of the C/N ratio. Nitrogen removal was affected by the C/N ratio. Indeed, at a C/N ratio equal to 10, nitrogen removal efficiency was maximum with an average removal of 96.54%, 86.1% for ammonium and total nitrogen, respectively. Conversely, with a C/N ratio of 4, removal rate dropped significantly. Indeed, the average ammonium and total nitrogen removal were of 82% and 53%, respectively. An important nitrogen removal rate was achieved when the operating DO concentration was maintained between 1.0 and 2.0 mg L⁻¹. The impact of the packing material and Mixed Liquor Suspended Solid (MLSS) concentrations on oxygen transfer was determined experimentally. In fact, up to 23% increase in the values of the mass transfer coefficient was observed after the addition of a packing material inside the reactor. However, an increase in the MLSS led to a decrease of the oxygen transfer coefficient.

Keywords

Dissolved oxygen concentration; C/N ratio; Integrated fixed-film activated sludge; packing material; oxygen transfer coefficient

1. Introduction

Nitrogen (N) and phosphorus (P) are necessary elements for the development of microorganisms. High concentrations of N and P lead to eutrophication and have detrimental effects on aquatic life [1]. Therefore, it is necessary to remove N and P from wastewater before its discharge to natural water reservoirs in order to protect this resource. Biological treatment processes are primarily used to remove nitrogen because of their high efficiency and low cost [2]. Generally, biological removal of nitrogen involves mainly the following two-step process: (1) aerobic nitrification of ammonia (NH_4^+-N) first to nitrite (NO_2^--N) and later to nitrate (NO_3^--N) and (2) anoxic denitrification of the NO_3^--N to gaseous nitrogen compounds (N_2)[3,4]. These two steps could be performed simultaneously in a single reactor leading to a simplification of the required equipment. Hence, this process has been labeled "Simultaneous Nitrification/Denitrification" or SND [5].

Observations at bench-scale [6] and full-scale [4] levels have demonstrated that SND is an attractive solution for the removal of nitrogen from various effluents [7].

Integrated Fixed-film Activated Sludge (IFAS), also known as hybrid biological reactor, has been increasingly used in recent years due to its numerous advantages such as (1) ease of operation, (2) smaller reactor size, (3) reduced sludge production[8,9] and (4) possibility to operate IFAS at much lower mixed liquor SRT (Sludge Retention Time) values than in activated sludge process configuration, still having high nitrification performance even at low temperatures [10].

This technology is a modification of the conventional activated sludge by integrating solid media (typically suspended plastic pieces or fixed synthetic mesh) into bioreactors. Hence, IFAS reactors combine both suspended sludge and attached growth biomass features[1,11].

1 The introduction of solid supports could provide a supplementary specific surface area for
2 biofilm growth. Hence, a suitable environment for aerobic and anoxic microorganisms in the
3 same reactor is achieved [7,12].
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6 Many types of supports have been studied thanks to their ability to improve the biological
7 treatment of nitrogen in IFAS processes. Eker et al. [13] used plastic brushes to improve the
8 performance of the system. They reported a high rate of COD and toxicity removal using this
9 system. This improvement is attributable to the microorganisms, which fixed on the brushes
10 and formed a biofilm. In Japan, the comparison between a system with the hollow
11 polypropylene pellets integrated into the anoxic reactor and the other without pellets was
12 conducted at a temperature of 15 ° C. It is reported that the use of packing allows high rates of
13 denitrification and ammonification, thus providing high efficiency of nitrogen removal [14].
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16 The DO concentration is an essential factor of a successful SND formation in the hybrid
17 biological reactor [15]. Indeed, this process, which combines nitrification and denitrification,
18 occurs naturally inside microbial flocs or biofilms as a result of the DO concentration
19 gradients present across the biomass [16]. Furthermore, in order to achieve a balanced
20 equilibrium between the nitrification and denitrification processes, the DO levels must be
21 appropriate and controlled inside the SND reactor [17].
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24 Xia et al. [18] have shown a high yields of SND when dissolved oxygen concentrations is
25 maintained between 1.5 and 2 mgL⁻¹.
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28 The aeration in these hybrid processes has two objectives: (1) the fluidization of solid media
29 in order to optimize the contact between pollutants and the biomass attached to the supports
30 and (2) supplying the oxygen needed for the growth of the aerobic biomass. Oxygenation
31 performance strongly depends on the characteristics of the solid phase (concentration and
32 size of solids) [19,20]. The aeration capacity of a reactor is characterized by the oxygen
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transfer coefficient called " $K_L a$ ". This overall transfer coefficient reflects the quality of the transfer of gas to a liquid.

" $K_L a$ " is accessible through experience. To determine the value of this coefficient, one can either measure $K_L a$ directly or measure K_L (the mass transfer coefficient) and a (the specific area of bubbles). However, it is difficult to independently measure K_L and a . In general, K_L is deduced from measuring $K_L a$ and a [21], measured by a chemical or optical method. However, the measurement of a is a source of error and it is preferable to measure $K_L a$ directly[22]. $K_L a$ varies depending on the hydrodynamics (type of reactor, geometry, operating conditions), the physical and chemical properties of the liquid (presence of surfactants, viscosity, the presence of solid particles, etc.) and the temperature. In addition, solid media used in hybrid process affect the oxygen mass transfer. The specific impact of solid media remains poorly understood both at the pilot [23,24] and at the industrial [25,26] scales. The IFAS configuration aims to reduce energy costs by increasing the oxygen transfer coefficient " $K_L a$ " compared to the conventional activated sludge system.

This paper aims to determine the influence of C/N ratio and DO concentration on an IFAS system, treating a synthetic wastewater, for the removal of carbon and nitrogen. We also assess the impact of the solid media and Mixed Liquor Suspended Solid (MLSS) concentrations on the rate of oxygen mass transfer through the evaluation of two quantities namely the alpha coefficient and $K_L a$.

2. Materials and methods

2.1. Experimental setup

All experiments were carried out on a pilot-scale fixed media IFAS reactor (Fig.1) operated under the conventional activated-sludge process configuration. The experimental setup consisted of the following components: feeding tank (volume 200L), reactor (32L of useful volume), settling tank (28L of useful volume), air compressor (the aeration flow rate was

maintained at 20 Lmin⁻¹), two peristaltic pumps; one supplies the substrate, while the other recycles the activated sludge, and two fine bubble diffusers placed at the bottom of the aeration basin to provide dissolved oxygen. The pilot plant also contained a cylindrical plastic brush attached to the inside of the reactor and served as a bacterial carrier [13].

The technical specifications of our pilot plant are listed in Table 1.

2.2. Wastewater and seed sludge

The synthetic wastewater which was prepared every two days, is composed of glucose (C₆H₁₂O₆), Ammonium sulfate (NH₄)₂SO₄ and Potassium dihydrogen phosphate (KH₂PO₄)₂ [27]. This mixture was continuously fed at flow rates between 1 and 1.5 Lh⁻¹. The activated sludge used for seeding was taken from the aeration basin of Ouardanine WasteWater Treatment Plant: WWTP (Tunisia). The initial MLSS concentration was of close to 10.0 g TTS L⁻¹. This activated sludge was diluted with tap water until reaching a MLSS concentration of about 4.0 gL⁻¹.

2.3. Physico-chemical analysis

Ammonia nitrogen (NH₄⁺-N)(Basic distillation followed by colorimetric assay), nitrate nitrogen (NO₃⁻-N)(Electrochemical method: nitrate ion selective electrode WTW- 106 624), COD and MLSS were analyzed in accordance with the standard methods for the examination of water and wastewater [28]. The pH and dissolved oxygen concentration were measured using a pH meter (Eutech, pH700) and an oxygen sensor (Endress+Hauser, Oxymax COS41), respectively.

2.4. Reactor operation

After seeding with activated sludge from the municipal WWTP of Ouardanine (Tunisia), the pilot plant was operated for 190 days without interruption.

The system was operated at the solid retention time (SRT) of 35 days, water temperature of 18-26°C and pH of 6.0-8.0.

Four operation cycles with different C/N ratios of the inlet wastewater were applied: Cycle 1, C/N= 12 (day 37–76); Cycle 2, C/N= 10 (day 77–116); Cycle 3, C/N= 8 (day 117–156); Cycle 4, C/N= 4 (day 157–190).

The period preceding the 4 cycles (i.e., day 1–36) was intended to acclimatise the activated sludge in the reactor to the synthetic substrate. This acclimatization process was considered to be complete only when the system yield became stable (i.e., when the carbon and nitrogen removal performances were stable).

During the operation, the COD volume load was set to $0.83 \text{ kg}_{\text{COD}}\text{m}^{-3}\text{day}^{-1}$, while that of nitrogen was increased gradually to determine the system's effectiveness as a function of Nitrogen pollution levels.

DO concentration was maintained in the reactor at a desired level using a controller for the aeration intensity. Dissolved Oxygen (DO) was closely monitored during the entire experimental period using the Oxymax COS41 oxygen sensor, linked to an electrical box, thus allowing to automatically control the stirring and the injection of air into the reactor.

The operating conditions are detailed in Table 2.

2.5. Oxygen transfer measurements

Oxygen transfer is evaluated by the product, $K_L a$, expressed in s^{-1} , where “a” is the specific area of bubbles, and “ K_L ” is the mass transfer coefficient [29].

2.5.1. Determination of oxygen transfer coefficient in clean water ($K_L a$)

The method used to determine “ $K_L a$ ” relies on a two-step: de-oxygenating/reoxygenating of the clean water contained in the system [30]. Initially, sodium sulfite was used in excess to decrease the oxygen dissolved in the water by the reaction [31]:

In order to enhance this reaction, a catalyst (e.g., cobalt) was introduced in the reactor. Therefore, the concentration of oxygen in the system rapidly dropped to zero.

When all the sulfites were totally oxidized, the oxygen brought by the aeration system was no longer consumed and its concentration increased until saturation. The measurement of dissolved oxygen concentration was reported every twenty seconds.

The differential equation given below represents the oxygen mass balance in clean water (in the absence of biomass) [32]:

$$\frac{dC_L}{dt} = K_L a (C_S - C_L) \quad (1)$$

Where

$\frac{dC_L}{dt}$ [mgL⁻¹s⁻¹] is the variation of DO concentration as a function of time

$K_L a$ [s⁻¹] refers to the oxygen transfer coefficient measured in clean water

C_S [mgL⁻¹] is the DO concentration at saturation in clean water

C_L [mgL⁻¹] represents the DO concentration in the liquid phase at time t

If we assume that the liquid phase (clean water) was homogeneous, the integration of equation 1 leads to:

$$\ln \left[\frac{C_S - C_0}{C_S - C_L} \right] = K_L a \cdot t \quad (2)$$

Where C_0 [mgL⁻¹] represents the DO concentration at t = 0

Therefore, $K_L a$ appears as the slope of the linear function relating $\ln \left[\frac{C_S - C_0}{C_S - C_L} \right]$ vs. time.

In water, the solubility of oxygen is temperature dependent [32]. Hence, one needs to convert the values determined at the temperature of the experiment to the standard temperature of T= 20°C using the following empirical equation [33]:

$$K_L a = K_L a_T 1.024^{(20-T)} \quad (3)$$

Where

$K_L a$ [s^{-1}] is the oxygen transfer coefficient at 20°C

$K_L a_T$ [s^{-1}] refers to the oxygen transfer coefficient at the test time, T

1.024 : The correction factor generally used when the temperature is between 15 to 35°C.

2.5.2. Determination of oxygen transfer coefficient in the presence of suspended solids ($K_L a'$)

The solubility of oxygen in a liquid is affected by several parameters such as temperature, pressure, salinity, viscosity and in particular the presence of particles in suspension. In this sense, it is important to measure $K_L a$ in the presence of sludge [19].

In order to investigate the biomass samples from pilot-scale fixed media IFAS reactor, different MLSS were employed.

In this test, the determination of $K_L a$ was carried out in the same way as in the clean water by de-oxygenating/reoxygenating, but in this case we did not add any chemical reagent. The decrease in the concentration of oxygen results from the respiration of microorganisms contained in the system. The oxygen mass balance is given by Eq (4) [30]:

$$\frac{dC_L}{dt} = K_L a' (C_s^* - C_L) \quad (4)$$

Where

$K_L a'$ [s^{-1}] is the oxygen transfer coefficient measured in the presence of suspended solids

C_s^* [mgL^{-1}] is the dissolved oxygen concentration at saturation in the presence of suspended solids

C_L [mgL^{-1}] is the oxygen concentration in the liquid phase at time t

Therefore, after the integration of equation (4), $K_L a'$ appears as the slope.

2.5.3. Determination of alpha coefficient (α)

We introduced a coefficient, α , to measure the departure of the oxygen transfer in the activated sludge from its counterpart in clean water at the same temperature. This alpha coefficient (α) is defined by the following ratio[34] :

$$\alpha = K_L a' / K_L a \quad (5)$$

Where

$K_L a$ [s^{-1}] is the oxygen transfer coefficient measured in the presence of suspended solids

$K_L a$ [s^{-1}] is the oxygen transfer coefficient measured in clean water

2.5.4. Effect of packing material

The purpose of this measure was to characterize the effect of packing material on the transfer rate and solubility of oxygen. The tests for measuring oxygen transfer in the presence of suspended solids were carried out with and without packing material according to the protocol described above (2.5.2).

The percentage increase in the oxygen transfer coefficient $K_L a$ was calculated using equation (6):

$$\text{Increasing } K_L a \text{ (\%)} = \frac{K_L a(\text{with packing material}) - K_L a(\text{without packing material})}{K_L a(\text{without packing material})} * 100 \quad (6)$$

2.6. Statistical analysis

Statistical analysis of the data was performed using the SPSS 21 software with a confidence interval of 95%. To verify the difference between the nitrogen removal efficiencies for the different C/ N ratios and for the different dissolved oxygen concentrations, Anova test followed by Tukey post hoc was performed. The effect of packing material was statistically verified using the Student test.

For all tests, the differences were considered significant only if $p < 0.05$.

3. Results and discussion

3.1. Influence of DO concentration

1 In this study, the effect of DO concentration on nitrogen removal performance was studied for
2 nearly a month. As shown in Fig. 2, the general trend of the curves indicates that the TN and
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4 $\text{NH}_4^+\text{-N}$ removal rates were significantly influenced ($p<0.05$) by the DO concentration.
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6 During the first phase (i.e., the DO concentration was maintained between 0.5 and 1.0
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8 mgL^{-1}), the average $\text{NH}_4^+\text{-N}$ and TN removal efficiencies obtained, were 73.7% (± 8.09 SD)
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10 and 67.9% (± 8.89 SD), respectively where SD was a standard deviation.
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14 The obtained $\text{NH}_4^+\text{-N}$ removal rate could therefore suggest that the limitation of nitrification
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16 was caused by low DO concentration. Indeed, when the DO was in this range, $\text{NH}_4^+\text{-N}$ was
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18 the principal form of nitrogen in the effluent corresponding to the average $\text{NH}_4^+\text{-N}$
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20 concentration of 19.66 mgL^{-1} (± 1.14 SD). We conclude that the ammonia oxidation may be
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22 hampered by the low DO concentration. Similarly, Campos et al. [35] reported that the
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24 ammonia oxidation was mainly accomplished at a DO concentration above 1 mgL^{-1} , while at
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26 a DO concentration of 0.6 mgL^{-1} , the oxidation of ammonia became slower, which led to the
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28 accumulation of ammonia.
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34 On the other hand, under similar conditions, the diffusion of oxygen in flocs was limited and
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36 zones where the sludge floc became anoxic and anaerobic zones appeared, resulting in an
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38 increase in the aerobic denitrification capacity of the hybrid system. Concurrently, the
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40 nitrifiers present in the system were also inhibited. This means that at low DO concentrations,
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42 the step of nitrification was slowed down, which hindered the $\text{NH}_4^+\text{-N}$ and TN removal via
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44 SND.
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49 In phase II, when DO was maintained between 1.0 and 2.0 mgL^{-1} , nitrification was
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51 significantly enhanced ($p<0.05$) compared to the result obtained during the first phase with an
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53 average of 91% (± 9.35 SD) and the TN removal efficiency was significantly higher
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55 ($p=0.008<0.05$), reaching 81.8% (± 9.08 SD)..
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As the DO was further increased to 3.0 mgL^{-1} (phase III), nitrification improved slightly but no significantly ($p=0.149 > 0.05$) compared to the second phase, with an average NH_4^+-N removal efficiency of 98% ($\pm 0.97 \text{ SD}$), with effluent NH_4^+-N concentration of 1.33 mgL^{-1} ($\pm 0.64 \text{ SD}$). However, this increase of DO led to a decrease in the TN removal (72%). Indeed, unlike the first phase, nitrates became the main product of nitrogen in the system SND($\text{NO}_3^- - \text{N}$ concentration was elevated from 6 mgL^{-1} to 18 mgL^{-1}). This indicates that this relatively high concentration of oxygen (i.e., 3 mgL^{-1}) has significantly inhibited the activity of denitrifiers. Indeed, this type of bacteria are more active in areas where the concentration of DO is very low or even close to zero [36]. This justifies the decrease of the denitrification capacity at higher concentrations of DO.

The effluent $\text{NO}_3^- - \text{N}$ concentration in IFAS reactor with a low or medium dissolved oxygen concentration ($0.5 < \text{OD} < 1$ or $1 < \text{OD} < 2$) was significantly ($p < 0.05$) lower than those with a high concentration ($\text{DO} > 3$). In contrast, the effluent NH_4^+-N concentration in this system with a low dissolved oxygen concentration ($0.5 < \text{OD} < 1$) were significantly ($p < 0.05$) higher than those with high or medium dissolved oxygen concentration.

In the IFAS system, despite the formation of an anoxic zone inside the biofilm, the denitrification process was not that high probably due to a lack of organic substances. Several studies have established the effect of oxygen concentration on the efficacy of SND [37]. In this study, the optimal value achieved for effective nitrogen removal (i.e., when DO was maintained between 1.0 and 2.0 mgL^{-1}) was consistent with the findings of Sriwiriyarat et al who showed that the optimal DO concentration in the IFAS system was 2 mgL^{-1} at C/N ratio of 10, unlike other studies that found lower DO concentration values [38].

Indeed, the optimal DO concentration, needed to achieve a maximum SND efficiency, depends on the adopted operating conditions. For instance, Meng et al (2008) suggested that the optimal concentration of dissolved oxygen was between 0.75 mgL^{-1} and 1.0 mgL^{-1} ,

1 while von Münch et al (1996) specified that an operating DO concentration of 0.5
2 mgL⁻¹ leads to a 100% SND efficiency. To conclude, one can state that low DO
3 concentrations result in better SND process performances.
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5 We conclude that the SND in the hybrid system is influenced by the variation of DO
6 concentration. Indeed, a high DO concentration leads to the limitation of ammonia oxidation,
7 whereas a low concentration of DO hinders denitrification.
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9 **3.2. Influence of biomass concentration on oxygen transfer**

10 Fig. 3 depicts the general trend of re-oxygenation curves in the mixed liquor containing
11 different concentrations of biomass (ranging from 2 to 10 gL⁻¹). For the different biomass
12 concentrations, this figure indicates that oxygen saturation was reached after approximately ten
13 minutes of aeration. Our findings clearly suggest that the increase of the biomass concentration
14 significantly affected ($p < 0.05$) the solubility of oxygen. When the concentration of biomass
15 was higher, the concentration at saturation was weaker and the time required to reach saturation
16 was longer.
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18 The objective of this manipulation was to evaluate the effect of MLSS concentrations on the
19 oxygen transfer characteristics (alpha coefficient (α) and K_La coefficient).
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21 Applying equation (2), we have plotted $\ln \left[\frac{C_s - C_0}{C_s - C_L} \right]$ as a function of time (Fig.4).
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23 So, K_La was obtained from the slope.
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25 An increase in MLSS led to a decrease in K_La (see Table 3). Therefore, the biomass with the
26 highest MLSS resulted in the lowest K_La values. Initially for each additional gL⁻¹, K_La
27 dropped about 10%. As the biomass concentration increased, this percentage decreased to
28 about 3%. Such a result is probably due to an obstruction of gas-liquid interfaces. Indeed, It
29 has been reported that such blockage is reflected by a decrease in the specific area of bubbles
30 owing to the adsorption of particles on the gas bubbles [30]. Hence, we conclude that biomass
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concentration should not exceed 6 gL^{-1} in the case of IFAS system to ensure optimal oxygen transfer efficiency. Indeed, higher values of the MLSS concentration lead to an excessive consumption of energy to pass little oxygen in solution.

The results depicted in Fig.5 show that an increase in MLSS concentration also leads to a decrease in the alpha coefficient. The variation of this coefficient was directly related to the presence of MLSS, which can alter the mass transfer coefficient by introducing additional resistance to oxygen transport [39]. For an MLSS concentration below 6.2 gL^{-1} , the alpha coefficient values decreased significantly. Further increase in MLSS concentration does not result in a significant decrease in the alpha coefficient. Moreover, previous studies using tanks of various geometries [34,40,41], showed the existence of an exponential correlation between the MLSS concentration and the alpha coefficient.

The solids concentration appears as the main parameter controlling simultaneously the alpha coefficient and $K_L a$. The effects of this parameter on oxygen transfer were established by various researchers [34,42,43].

In the literature, there is some disagreement on the influence of the presence of suspended solids on oxygen transfer. Casey et al. [44], however, note that the discrete suspended solids have no influence on oxygen transfer for $0 < X < 10 \text{ gL}^{-1}$. In contrast Germain et al. [34] noted an influence of concentration on alpha coefficient and $K_L a$ in a concentration range of 7.2 to 30.2 gL^{-1} .

For the present study, oxygen transfer can be enhanced by using a low concentration of solids (i.e., MLSS concentrations less than $< 6 \text{ gL}^{-1}$). Germain et al (2007) found that MLSS concentration should be $< 10\text{--}15 \text{ gL}^{-1}$ to have the best efficiency of oxygen transfer.

3.3. Effect of the packing material (plastic brush) on oxygen transfer

Few studies have dealt with the effect of the presence of a packing material on oxygen transfer [24,26]. Indeed, the impact of the packing material on oxygen transfer in IFAS

1 reactor is of paramount importance. To better understand this phenomenon, we have
2 conducted a set of tests to measure the oxygen transfer coefficient for different MLSS
3 concentrations.
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6 Fig. 6 displays the evolution of the oxygen transfer coefficient at various MLSS concentrations.
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8 Clearly, the presence of a packing material substantially improved the coefficient of oxygen
9 transfer (K_La) regardless of the MLSS concentration used. Statistical analysis proved that the
10 K_La obtained in IFAS reactor was significantly higher ($p < 0.05$) than the case with the reactor
11 without a packing material. As an example, K_La increased by about 23% with an MLSS
12 concentration of 3 gL^{-1} . This improvement in K_La detected in the case of IFAS reactor was due
13 to the presence of a packing material in the reactor which breaks up the largest air bubbles into
14 smaller ones [45]. In fact, the larger interfacial area “a” between liquid and gas greatly enhance
15 the gas/liquid mass transfer coefficient [30]. In addition, the presence of the packing material
16 most likely increases the residence time of the air bubbles in wastewater [46].
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18 Conversely, in the case of a conventional reactor (i.e., without packing), the air bubble is driven
19 through the wastewater by the buoyant force of air which favors the increase of the air bubble
20 diameter and therefore the reduction of the interfacial area [30].
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23 **3.4. Removal performance under different C/N ratios**

24 **3.4.1. COD removal**

25 Fig. 7 provides a graphical representation of both the COD concentration profiles in the
26 effluent and the achieved removal efficiencies at various C/N ratios and for the entire operation
27 period starting after the acclimatization. Clearly, an excellent COD removal was achieved.
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29 The biological removal of COD showed a stable trend, reaching values higher than 90%. This
30 is due to the good biological activity within the system, which is due to the biological
31 acclimatization to wastewater features.
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Various influent COD concentrations (i.e., ranging from 400mgL⁻¹ to 1300mgL⁻¹) were used. Nonetheless, the largest effluent COD concentration observed was 129.4 mgL⁻¹ for a concentration of 1200 mgL⁻¹. The average removal efficiencies were 93.07% (±2.64 SD), 96.01% (±1.29 SD), 95.53% (±1.12 SD) and 95.79% (±1.4 SD), respectively for C/N ratio of 12, 10, 8 and 4. All the values of these efficiencies were very large, which suggests that the investigated C/N ratios have only a minor effect on the COD removal. This is because we have used a readily biodegradable synthetic substrate (i.e., glucose). Hence, whatever the operating condition, the efficiency of COD removal turn out to be very high. Finally, it is worth noting that our results of removal efficiencies obtained using the IFAS reactor are in accordance with other results obtained using SND reactors [36,47,48].

3.4.2. Nitrogen removal

The average values of nitrogen removal under different influent C/N ratios were not as good as those of organic matter removal, as shown in Fig. 8.

When C/N ratio was maintained at 12, the average NH₄⁺-N and TN removal efficiencies were 88.2% (±10.84 SD) and 74.6% (±9.35 SD), respectively. The effluent NH₄⁺-N concentration was 8.53 mgL⁻¹(±7.44 SD). Good nitrification was achieved following the development and enrichment of the nitrifiers after the acclimatization stage.

During the second cycle (C/N = 10), from 77 d to 116 d, the system showed a significant performance of nitrogen removal. Indeed, the nitrification activity increased gradually, and the average rate of NH₄⁺-N removal was 96.54% (±2.44 SD) with an effluent NH₄⁺-N concentration of 3.32 mgL⁻¹(±2.96 SD). The nitrification efficiency was significantly higher (p < 0.05) than the case when C/N ratio was maintained at 12. The observed important efficiency was most likely due to the development of a biofilm in the reactor, which is characterized by a high retention time on the carriers [48]. However, during this phase, a higher value of nitrification was achieved compared to other studies, such as that of Liu et al.

[49], who found that a C/N ratio higher than 5 promotes a significant decrease of the nitrification efficiency.

TN removal efficiency obtained in the second cycle (i.e., 10) with an average value of 86.1% (± 2.61 SD) was significantly ($p < 0.05$) higher than that of the first cycle (i.e., 12). This means that a highly efficient denitrification was achieved under this condition thanks to the promotion of nitrification. TN removal efficiency mostly depended on the nitrification efficiency in the system. Such a result could be explained by the development and enrichment of heterotrophic microorganism populations due to the abundant organic carbon source [50]. Hence, this result suggests that with a C/N ratio of 10, the nitrification and denitrification efficiencies were strengthened.

During the third cycle (C/N = 8), from day 171 to day 156, average efficiency of nitrification was 95% (± 3.31 SD) corresponding to an average $\text{NH}_4^+ - \text{N}$ concentration of 5.39 mgL^{-1} (± 3.89 SD), with no significant ($p = 0.795 > 0.05$) statistically difference in ammonium removal between the two ratio C/N (10 and 8). However, the TN removal efficiency dropped to 77% (± 8.11 SD). This significant decrease ($p = 0.000061 < 0.05$), from 86% in the second cycle to nearly 77% in the third one, maybe due to carbon substrate limitation.

In cycle 4, with a C/N ratio maintained at 4, the $\text{NH}_4^+ - \text{N}$ removal was always at a high level with an average value of 82% (± 3.54 SD) with an effluent $\text{NH}_4^+ - \text{N}$ concentration of 38.87 mgL^{-1} (± 9.03 SD). This reduction was moderate (i.e., from 95% to 82%) but significantly ($p < 0.05$) lower than other cycles. The nitrification rate was, therefore, always greater than 80% when the C/N ratio was higher than 4. This is probably due to the formation of a biofilm inside the which has become more stable in the face of external perturbation after 156 days of operation of the hybrid system (i.e., IFAS Reactor).

These obtained performances are different from those observed by Mannina et al. [48] who found an average of 80% nitrification for a C/N ratio equal to 2.

Conversely, as shown in Fig. 8, the important C/N decrease caused a significant decrease in the denitrification rate ($p<0.05$) than others cycles. Indeed, the TN removal efficiency dropped to 53% (± 10.53 SD). This phenomenon could be explained by the low denitrification when the organic compounds are limiting for the denitrifiers. Thus, the activity of the denitrifying bacteria is inhibited.

Moreover, with a low C/N ratio (i.e., equal to 4), the heterotrophic bacteria responsible for denitrification do not proliferate more rapidly, which leads to a decrease in oxygen consumption.

This bacterial laziness automatically increased the amount of oxygen in the reactor. Thus, it hindered the denitrification process which requires an aerobic or even anoxic environment.

However, these conditions caused the accumulation of nitrates and consequently restrained the SND process. At a low C/N ratio, a large decrease in denitrification efficiency is also reported by Matsumoto et al. [51].

Zhang et al. [50] have shown that when nitrification and denitrification occur simultaneously in the same reactor, it is essential to control the balance between these two processes.

Based on experimental results, a higher nitrogen removal efficiency is obtained when the substrate C/N ratio is maintained at 10.

4. Conclusion

In the present study, we highlighted the effect of the DO concentration and C/N ratio on the removal of organic carbon and nitrogen using the IFAS system.

Our experimental measurements indicate that Nitrogen removal performance greatly depends on the C/N ratio. Indeed, the nitrification and denitrification rates were very important during the second cycle when the C/N ratio was fixed at 10. Under this condition, we observed the best results in terms of biological removal of carbon and nitrogen. During cycle 4, the decrease of C/N ratio (i.e., down to 4) generated a significant decrease of nitrogen removal. This in turn led to a dramatic reduction of TN removal as a result of the simultaneous decrease of nitrification and denitrification.

Our experiments indicated that both high and low concentrations of DO have negative effects on the efficiency of the SND process in an IFAS reactor. The optimum SND is achieved with a DO concentration maintained between 1.0 and 2.0 mgL⁻¹.

Finally, both the $K_L a$ and α coefficient both increased with the addition of a packing material and decreased with increasing MLSS.

Acknowledgements

The authors would like to thank the Tunisian Ministry of Higher Education and Scientific Research for the financial support.

REFERENCES

- [1] Y. Shao, Y. Shi, A. Mohammed, Y. Liu, Wastewater ammonia removal using an integrated fixed-film activated sludge-sequencing batch biofilm reactor (IFAS-SBR): Comparison of suspended flocs and attached biofilm, *Int. Biodeterior. Biodegrad.* 116 (2017) 38–47.
- [2] J. Duan, H. Fang, B. Su, J. Chen, J. Lin, Characterization of a halophilic heterotrophic nitrification-aerobic denitrification bacterium and its application on treatment of saline wastewater, *Bioresour. Technol.* 179 (2015) 421–428.
- [3] Y.H. Ahn, Sustainable nitrogen elimination biotechnologies: A review, *Process. Biochem.* 41 (2006) 1709–1721..
- [4] X. Zhou, Y. Han, X. Guo, Identification and evaluation of SND in a full-scale multi-channel oxidation ditch system under different aeration modes, *Chem. Eng. J.* 259 (2015) 715–723.
- [5] X.J. Wang, S.Q. Xia, L. Chen, J.F. Zhao, N.J. Renault, J.M. Chovelon, Nutrients removal from municipal wastewater by chemical precipitation in a moving bed biofilm reactor, *Process Biochem.* 41 (2006) 824–828.
- [6] Y. Liu, H. Shi, L. Xia, H. Shi, T. Shen, Z. Wang, G. Wang, Y. Wang, Study of operational conditions of simultaneous nitrification and denitrification in a Carrousel oxidation ditch for domestic wastewater treatment, *Bioresour. Technol.* 101 (2010) 901–906.
- [7] S. Yang, F. Yang, Z. Fu, R. Lei, Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on organic carbon and nitrogen removal, *Bioresour. Technol.* 100 (2009) 2369–2374.
- [8] N.K. Singh, A.A. Kazmi, M. Starkl, Environmental performance of an integrated fixed-film activated sludge (IFAS) reactor treating actual municipal wastewater during start-up phase, *Water Sci. Technol.* 72 (2015) 1840–1850.
- [9] P. Regmi, W. Thomas, G. Schafran, C. Bott, B. Rutherford, D. Waltrip, Nitrogen removal assessment through nitrification rates and media biofilm accumulation in an IFAS process demonstration study, *Water Res.* 45 (2011) 6699–6708.
- [10] D. Di Trapani, M. Christensson, M. Torregrossa, G. Viviani, H. Ødegaard, Performance of a hybrid activated sludge / biofilm process for wastewater treatment in a cold climate region : Influence of operating conditions, *Biochem. Eng J.* 77 (2013) 214–219.
- [11] H. su Kim, J.W. Gellner, J.P. Boltz, R.G. Freudenberg, C.K. Gunsch, A.J. Schuler, Effects of integrated fixed film activated sludge media on activated sludge settling in biological nutrient removal systems, *Water Res.* 44 (2010) 1553–1561.
- [12] Y. Watanabe, S. Masuda, M. Ishiguro, Simultaneous nitrification and denitrification in micro-aerobic biofilms, *Water Sci. Technol.* 26 (1992) 511–522.
- [13] S. Eker, F. Kargi, Biological treatment of para -chlorophenol containing synthetic wastewater using rotating brush biofilm reactor, *J. Hazard. Mater.* 135 (2006) 365–371.
- [14] S. Takizawa, V. Aravinthan, K. Fujita, Nitrogen removal from domestic wastewater using immobilized bacteria, *Water Sci. Technol.* 34 (1996) 431–440.

- [15] W. Ma, Y. Han, W. Ma, H. Han, H. Zhu, C. Xu, K. Li, D. Wang, Enhanced nitrogen removal from coal gasification wastewater by simultaneous nitrification and denitrification (SND) in an oxygen-limited aeration sequencing batch biofilm reactor, *Bioresour. Technol.* 244 (2017) 84–91.
- [16] E. V. Münch, P. Lant, J. Keller, Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors, *Water Res.* 30 (1996) 277–284.
- [17] Y.C. Chiu, L.L. Lee, C.N. Chang, A.C. Chao, Control of carbon and ammonium ratio for simultaneous nitrification and denitrification in a sequencing batch bioreactor, *Int. Biodeterior. Biodegrad.* 59 (2007) 1–7.
- [18] S. Xia, J. Li, R. Wang, Nitrogen removal performance and microbial community structure dynamics response to carbon nitrogen ratio in a compact suspended carrier biofilm reactor, *Ecol. Eng.* 32 (2008) 256–262.
- [19] A. Ferreira, C. Ferreira, J.A. Teixeira, F. Rocha, Temperature and solid properties effects on gas–liquid mass transfer, *Chem. Eng. J.* 162 (2010) 743–752.
- [20] P. Mena, A. Ferreira, J.A. Teixeira, F. Rocha, Effect of some solid properties on gas–liquid mass transfer in a bubble column, *Chem. Eng. Process. Process Intensif.* 50 (2011) 181–188.
- [21] K. Van't Riet, Review of measuring methods and results in nonviscous gas-liquid mass transfer in stirred vessels, *Ind. Eng. Chem. Process. Des. Dev.* 18 (1979) 357–364.
- [22] V. Linek, P. Benes, V. Vacek, Dynamic pressure method for k_La measurement in Large-scale bioreactors, *Biotechnol. Bioeng.* 33 (1988a) 1406–1412.
- [23] H. Pham, S. Viswanathan, R.F. Kelly, Evaluation of Plastic Carrier Media Impact on Oxygen Transfer Efficiency with Coarse and Fine Bubble Diffusers, *Proc. Water Environ. Fed. 2008* (2008) 5069–5079.
- [24] J.Y. Jing, J. Feng, W.Y. Li, Carrier effects on oxygen mass transfer behavior in a moving-bed biofilm reactor, *Asia-Pacific J. Chem. Eng.* 4 (2009) 618–623.
- [25] S. Viswanathan, H. Pham, R.F. Kelly, D.T. Redmon, W. Fernandes, Evaluation of Oxygen Transfer Efficiency via Off-gas Testing at Full Scale Integrated Fixed film Activated Sludge Installation, *Proc Water Environ. Fed. 2008* (2008) 4994–5001
- [26] D. Rosso, S.E. Lothman, M.K. Jeung, P. Pitt, W.J. Gellner, A.L. Stone, D. Howard, Oxygen transfer and uptake, nutrient removal, and energy footprint of parallel full-scale IFAS and activated sludge processes, *Water Res.* 45 (2011) 5987–5996.
- [27] D. Ding, C. Feng, Y. Jin, Effect of C / N Ratio on Nitrogen Removal in a Novel Sequencing Batch Biofilm Reactor, 4th International Conference on Bioinformatics and Biomedical Engineering., Chengdu, China, 2010.
- [28] Apha., Awwa., Wef, Standards Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, 1998.
- [29] F. Garcia-ochoa, E. Gomez, Bioreactor scale-up and oxygen transfer rate in microbial processes : An overview, *Biotechnol. Adv.* 27 (2009) 153–176.

- [30] K. Zerari, I. Seyssieq, D.E. Akretche, N. Roche, Enhancement of oxygen mass transfer coefficients in a hybrid membrane bioreactor, *J. Chem. Technol. Biotechnol.* 88 (2013)
- [31] C.M. Cooper, G.A. Fernstorm, S.A. Miller, Performance of agitated gas-liquid contactors, *Ind. Eng. Chem.* 36 (1944) 504-509
- [32] A.E. Ghaly, A Microcomputer-Based Oxygen Measurement and Control System for Fermentation Processes, *Biotechnol. Appl. Biochem.* 30 (1991) 247-263.
- [33] M.K. Stenstrom, R.G. Gilbert, Effects of alpha, beta and theta factor upon the design, specification and operation of aeration systems, *Water Res.* 15 (1981) 643-654.
- [34] E. Germain, F. Nelles, A. Drews, P. Pearce, M. Kraume, E. Reid, S.J. Judd, T. Stephenson, Biomass effects on oxygen transfer in membrane bioreactors, *Water Res.* 41 (2007) 1038-1044.
- [35] J.L. Campos, J.M. Garrido, A. Mosquera-Corral, R. Méndez, Stability of a nitrifying activated sludge reactor, *Biochem. Eng. J.* 35 (2007) 87-92.
- [36] Q. Meng, F. Yang, L. Liu, F. Meng, Effects of COD/N ratio and DO concentration on simultaneous nitrification and denitrification in an airlift internal circulation membrane bioreactor, *J. Environ. Sci.* 20 (2008) 933-939.
- [37] V.S. Ruys, K. Zerari, I. Seyssieq, N. Roche, Study of Carbonaceous and Nitrogenous Pollutant Removal Efficiencies in a Hybrid Membrane Bioreactor, *J. Chem.* 2017 (2017) 1-7.
- [38] T. Sriwiriya, W. Ungkurarat, P. Fongsatitkul, S. Chinwetkitvanich, Effects of dissolved oxygen on biological nitrogen removal in integrated fixed film activated sludge (IFAS) wastewater treatment process, *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* 43 (2008) 518-527.
- [39] B. B. Ozbek, S. Gayik, The studies on the oxygen mass transfer coefficient in a bioreactor, *Process Biochem.* 36 (2001) 729-741.
- [40] E.B. Muller, A.H. Stouthamer, H.W. van Verseveld, D.H. Eikelboom, Aerobic domestic waste water treatment in a pilot plant with complete sludge retention by cross-flow filtration, *Water Res.* 29 (1995) 1179-1189.
- [41] J. Krampe, K. Krauth, Oxygen transfer into activated sludge with high MLSS concentrations, *Water Sci. Technol.* 47 (2003) 297-303.
- [42] L.K. Ju, A. Sundararajan, The effects of cells on oxygen transfer in bioreactors, *Bioprocess Eng.* 13 (1995) 271-278.
- [43] C. Freitas, J.A. Teixeira, Oxygen mass transfer in a high solids loading three-phase internal-loop airlift reactor, *Chem. Eng. J.* 84 (2001) 57-61.
- [44] T.J. Casey, O.T. Karmo, The influence of suspended solids on oxygen transfer in aeration systems, *Water Res.* 8 (1973) 805-811.
- [45] N. Epstein, Three-phase fluidization: Some knowledge gaps, *Can. J. Chem. Eng.* 59 (1981) 649-657.
- [46] S. Gillot, S. Capela, A. Heduit, Effect of horizontal flow on oxygen transfer in clean

water and in clean water with surfactants, *Water Res.* 34 (2000) 678–683.

- [47] B. Fu, X. Liao, L. Ding, H. Ren, Characterization of microbial community in an aerobic moving bed biofilm reactor applied for simultaneous nitrification and denitrification, *World J. Microbiol. Biotechnol.* 26 (2010) 1981–1990.
- [48] G. Mannina, G.A. Ekama, M. Capodici, A. Cosenza, D. Di Trapani, H. Ødegaard, Moving bed membrane bioreactors for carbon and nutrient removal: The effect of C/N variation, *Biochem. Eng. J.* 125 (2017) 31–40.
- [49] H. Liu, F. Yang, S. Shi, X. Liu, Effect of substrate COD/N ratio on performance and microbial community structure of a membrane aerated biofilm reactor, *J. Environ. Sci.* 22 (2010) 540–546.
- [50] Z. Zhang, Y. Li, S. Chen, S. Wang, X. Bao, Simultaneous nitrogen and carbon removal from swine digester liquor by the Canon process and denitrification, *Bioresour. Technol.* 114 (2012) 84–89.
- [51] S. Matsumoto, A. Terada, S. Tsuneda, Modeling of membrane-aerated biofilm: Effects of C/N ratio, biofilm thickness and surface loading of oxygen on feasibility of simultaneous nitrification and denitrification, *Biochem. Eng. J.* 37 (2007) 98–107.

Figure Caption

Fig. 1 The configuration of pilot-scale IFAS systems

Fig. 2 Profiles of NH_4^+ , NO_3^- and TN removal efficiency when the DO concentration is maintained between 0.5 and 1 (phase I), 1 and 2 (phase II), 2 and 4 (phase III)

Fig. 3 Oxygenation curves in the mixed liquor with different concentrations of biomass

Fig. 4 Determination of K_La

Fig. 5 Effect of biomass concentration on alpha coefficient

Fig. 6 Evolution of the oxygen transfer coefficient as a function of the different MLSS concentration (With and without packing material)

Fig. 7 COD concentration in the influent, effluent and COD removal efficiency

Fig. 8 NH_4^+ and TN removal under different COD/N ratios

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Table caption

Table 1	Technical design details of pilot plant
Table 2	Operating conditions applied in the IFAS reactor
Table 3	Summary of experimental results of oxygen transfer coefficient obtained at 20°C

Table 1 Technical design details of pilot plant

Parameters	Unit	Value
Dimension of reactor (¹ r * ² h)	cm	(17*45)
Reactor (volume)	L	40
Settling tank (useful volume)	L	28
¹ r: radius, ² h: height of the side		

Parameters	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	Average	SD	Average	SD	Average	SD	Average	SD
¹ C/N [-]	12	-	10	-	8	-	4	-
Substrate feed rate [L h ⁻¹]	1.19	0.17	1.16	0.18	1.16	0.18	1.16	0.19
Dissolved oxygen [mg L ⁻¹]	(0.5-1;1-2;2-3)	-	1-2	-	1-2	-	1-2	-
Average ² COD _{inlet} [mg L ⁻¹]	916	268	932.9	257	924.1	243	932.1	270
Average Total Nitrogen _{inlet} [mg L ⁻¹]	80.4	21	96.8	26	120.2	31	231.5	62
³ HRT[h]	27	3.9	28	4.2	28	4.1	28	4.3
Duration [d]	40	-	40	-	40	-	34	-

¹C/N: Carbon to nitrogen ratio, ²COD: Chemical oxygen demand, ³HRT: hydraulic retention time

Table 3 Summary of experimental results of oxygen transfer coefficient obtained at 20°C

[illegible]