A membrane assisted hybrid bioreactor for the post treatment of an anaerobic effluent from a fish canning factory

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Abstract An innovative membrane assisted hybrid bioreactor was used to treat a mixture of two streams produced in a fish canning factory: a highly loaded stream that had previously been treated in an anaerobic contact reactor, and a second stream with a relatively low COD and N concentration. Experiments were carried out during two experimental stages: an aerobic stage, which is focused in the study on the aerobic oxidation of ammonia and COD and a nitrification-denitrification stage in which the study was mainly focused on the removal of nitrogen. Results of the aerobic period pointed out that it was feasible to achieve ammonia and COD removals of around 99% at OLR of 6.5 kg COD/m³·d and NLR of 1.8 kg N-NH₄⁺/m³·d. Specific nitrifying activities of up to 0.78 g N-NH₄⁺/g protein·d and 0.25 g N-NH₄⁺/g VSS·d, were recorded for the attached and suspended biomass, respectively. Around 50–60% of the nitrifying capacity of the reactor was a result of the nitrifying capacity of the biofilm. During the nitrification-denitrification stage 76% of nitrogen removal was attained at an NLR of 0.8 kg N-NH₄⁺/m³·d. The biofilm nitrifying activity was not affected by the operating conditions of the system, as a result of the preferential consumption of COD by suspended biomass in the reactor. Thus, the combination of a hybrid system, with both suspended and attached biomass, and an ultrafiltration membrane module might be an alternative for treating wastewaters in compact biological systems. The intrinsic characteristics of the system made it feasible to operate at high OLR without problems related with the settling properties of the sludge or the drop in the nitrogen conversion. There were no solids in the effluent as a result of the use of the membrane filtration module.

Keywords Biofilm; denitrification; fish canning; hybrid reactor; nitrification; ultrafiltration membrane

Introduction
Seafood processing industries generate large quantities of highly polluted wastewaters with high concentrations of COD, solids, fats and protein (Veiga et al., 1994). These effluents exert a strong impact on the environment, being especially relevant when they are discharged into rivers, lakes or estuaries. Anaerobic digestion is an economical alternative to treat these effluents, which has been used during the last 15 years to treat these kinds of wastewaters. In spite of the high efficiency for COD removal, nitrogen removal percentage is almost negligible. Furthermore, COD concentration of the anaerobic effluents may still be high. Therefore, post-treatment of the anaerobic effluents is required in order to discharge them into the water body receptor, and to fulfil the legal requirements.

An economical alternative to remove the remaining COD and nitrogen fractions of the anaerobic effluents is the treatment of the waters using nitrification-denitrification biological processes. Usually, this is accomplished in activated sludge systems, in which the Organic Loading Rate (OLR) and the Nitrogen Loading Rate (NLR) are limited to around 1–2 kg COD/m³·d and 0.1 kg N/m³·d, respectively. This might be a problem for most of the fish canning factories, in which there is a lack of land for the installation of such a voluminous wastewater treatment system, as occurs in many Spanish facilities.

An alternative for the treatment in activated sludge systems is the utilization of high capacity biological systems. Hybrid suspended biomass-biofilm systems could be an
alternative. These systems have been successfully used not only to upgrade low nitrifying capacity wastewater treatment plants, but also as a new technology to develop compact systems for simultaneous nitrogen and organic matter removal. Some examples of them at lab scale and full scale are: the Pegasus system (Emori et al., 1996); the biolift reactor (Badot et al., 1994), the hybrid biofilm Airlift Suspension – Activated sludge reactor (van Benthum et al., 1998), an activated sludge system in which plastic support was added to the reactor in order to promote nitrification (Münch et al., 2000), and a hybrid Circulating Bed Reactor (HYCIFLO) developed in our laboratory (Oyanedel et al., 2000). All these hybrid systems use settlers as solids separation systems. A problem of hybrid systems with a settler for biomass separation is related with the settling properties of the sludge generated. For these systems the settleability of the sludge decreases when OLR is increased (Odegaard et al., 2000), which limits the OLR that could be applied for many of these hybrid systems. This limitation could be overcome by replacing settlers with membrane filtration units. Membrane filtration is an emergent technology, which will improve the liquid solid separation, allowing total control of the solid retention time inside the bioreactor. Besides, the effluent quality, both in terms of composition and bacteriological quality is better than in conventional sedimentation systems.

The main objective of this work is to present the results obtained during the operation of a membrane assisted hybrid system, with a predenitrification chamber, which was used to treat a mixture of two streams produced in a fish canning factory. Study of the evolution of the nitrifying capacity of the reactor, the possible effects of the suspended or colloidal COD in the behaviour of the unit and the biomass activity of both suspended biomass and biofilms were also topics that deserved special consideration.

**Materials and methods**

**Experimental set-up**

The system consisted of an anoxic chamber with suspended biomass (5.7 L) followed by an aerobic Circulating Bed Reactor (CBR) of 5.5 L which contains biofilm and suspended biomass. The aerobic reactor was coupled to a hollow fibre ultrafiltration membrane module (Zenon, ZW-1) contained in a vessel of 1.2 L which separated the permeate (effluent) from the retentate (sludge) which was recycled to the anoxic chamber (Figure 1). The membrane module characteristics are: average pore size of 0.045 µm, nominal surface area of 0.093 m² and typical operating transmembrane pressure of 10–50 kPa. This membrane is a flexible tube that resembled a thin macaroni noodle. Filtration is carried out from outside to inside. Air is injected continuously to clean the outer membrane surface. The rising flow of the air water mixture produces cleaning effects along the membrane surface (Engelhardt et al., 1998).

A granular product of high density polyethylene with a density of 0.9 g/cm³, constituted by particles with a size between 1 to 3 mm, was used as support for biofilm growth in the aerobic reactor. The carrier hold up in this reactor was 20% . Other characteristics of the CBR can be found in the literature (Lazarova and Manem, 1996; Garrido et al., 2000).

**Analytical methods**

pH and dissolved oxygen (DO) were determined by using specific electrodes. Nitrogen compounds (ammonia, nitrite and nitrate) in the liquid phase were measured daily by using spectrophotometric methods. COD was measured using a titrimetic method (APHA, 1998).

**Biomass concentration and specific activities**

The biomass concentration was measured periodically in both the biofilm and suspension in terms of COD, proteins, and polysaccharides. The suspended biomass was also
measured in terms of Volatile Suspended Solids (VSS). Determination of the nitrifying and heterotrophic activities, in the biofilm and the flocs, was done by Biological Oxygen Monitoring (Lazarova and Manem, 1996; Garrido et al., 2000). Denitrifying activities were determined by using samples of biomass from the reactor, which were disposed in tightly closed bottles containing a solution of nitrate and acetate.

**Operational conditions**

A concentrated stock medium (with the following concentrations: total COD between 1.8 and 2.1 g/L, soluble COD between 0.8 and 1 g/L, TKN around 0.4 g/L, N-NH\(_4^+\) around 0.38 g/L and DOC around 0.41 g/L) was obtained by mixing the influent and effluent of an industrial anaerobic contact reactor which treats a highly loaded stream of the factory. The stock medium was adequately diluted with tap water, in order to simulate the composition and characteristics of the water produced by the mixture of two streams of wastewater generated in the factory: the highly loaded one which was previously pre-treated in the anaerobic reactor and a low loaded stream with low COD and N concentration. The COD/N ratio of this mixture was around 5. The concentration of Cl\(^-\) was low, around 200 mg/L, as tuna processing in this factory was carried out using exclusively potable water and not seawater as occurred in many other factories in Spain.

The operation of the unit was divided into two different stages: an aerobic stage, and a nitrifying-denitrifying stage. During the aerobic stage the oxidation of organic matter and ammonia was studied while during the nitrifying-denitrifying stage the research was focused especially on the nitrogen removal. Table 1 shows the loading rates applied during

![Figure 1 Scheme of the predenitrification – nitrification system (1) anoxic chamber, (2) aerobic reactor, (3) membrane module, (4) manometer, (5) timer](image_url)

**Table 1** Operational conditions of the system during the two stages. *ALR referred to aerobic volume

<table>
<thead>
<tr>
<th>Period</th>
<th>Days</th>
<th>ALR* (kg N-NH(_4^+)/m(^3)-d)</th>
<th>OLR (kg COD/m(^3)-d)</th>
<th>Period</th>
<th>Days</th>
<th>ALR* (kg N-NH(_4^+)/m(^3)-d)</th>
<th>OLR (kg COD/m(^3)-d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0–18</td>
<td>0.22</td>
<td>1.1</td>
<td>6</td>
<td>99–143</td>
<td>1.7</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>19–35</td>
<td>0.50</td>
<td>2.5</td>
<td>7</td>
<td>144–166</td>
<td>0.85</td>
<td>4.25</td>
</tr>
<tr>
<td>3</td>
<td>36–56</td>
<td>1.0</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>57–70</td>
<td>1.0</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>71–98</td>
<td>1.7</td>
<td>8.5</td>
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the seven periods into which the two stages were divided. During these periods, different loading rates or concentrations were fed to the operating system, in order to check the behaviour of the unit.

Results and discussion

Aerobic stage (days 0–98)

During this stage, the anoxic chamber was not utilised and experiments were carried out in a system composed of the aerobic CBR coupled in series to the membrane module. During the first 57 operational days and the first 3 periods, ALR was stepwise increased from 0.22 to 1.0 kg N-NH$_4^+$/m$^3\cdot$d maintaining an ammonia concentration of 50 mg N-NH$_4^+$/$L$ and a Hydraulic Retention Time (HRT) of 1.3 h. From day 57 on, HRT was increased to 2.6 h increasing the ammonia concentration in the influent to 100 mg N-NH$_4^+$/$L$ and ALR up to 1.7 kg N-NH$_4^+$/$m^3\cdot$d. SRT was maintained for 50 days by purging between 180 and 250 mL of sludge.

The results showed that the reactor achieved gradually full ammonia conversion to nitrate, the COD removal being higher than 98%. There were temporary drops of ammonia conversion during the first days of each period, after stepwise increasing the loading rate of the system, but ammonia conversion was recovered a few days later. During the whole experiment, the hybrid reactor showed a high stability. The final COD and ammonia concentrations in the effluent were 110 mg/L and 1 mg N-NH$_4^+$/$L$, respectively (Figures 2 and 3). The results showed a higher ammonia removal percentage than a similar hybrid system which was used during previous research with a settler instead of the ultrafiltration module (Oyanedel et al., 2000). The better performance of the hybrid membrane assisted reactor can be explained by the substrate competition of suspended and attached micro-organisms, because suspended sludge in the membrane system presents a nitrifying activity which was negligible in a previous study using a hybrid system with settler (Oyanedel, 2002).

The biofilm concentration at the end of this stage was 3,000 mg COD/L. The biofilm specific nitrifying activity was around 0.68 g N-NH$_4^+$/$g$ protein$\cdot$d during the whole experimental period, and was not affected either by the ALR or the NLR of the system. The reason for such behaviour probably was a result of the preferential consumption of COD by the suspended flocs in the reactor. This made the consumption of most of the diffused oxygen by the nitrifiers in the biofilm and not by the heterotrophs in the biofilm feasible.

Figure 2 Evolution of the nitrogen compounds in the aerobic reactor: N-NH$_4^+$ influent (■); N-NH$_4^+$ effluent (○); N-NO$_3^-$ effluent (△)
Moreover, the nitrifying activity was also not affected by the presence of suspended or colloidal organic matter in the influent. The nitrifying activity was similar to those attained in previous research (Oyanedel, 2002), in which the same membrane system was fed with soluble organic matter as the only COD source. Probably in the hybrid system the suspended organic matter was absorbed or entrapped by the suspended flocs, protecting nitrifiers in the biofilm from the influence of these compounds. On the other hand, the heterotrophic specific activity in the biofilm was lower than 1.5 g O₂/g protein-d.

The suspended biomass concentration increased progressively with time till 4.8 g/L VSS at the end of the first stage, and did not achieve a stationary state. Solid retention time was 35 d and the biomass yield 0.22 g VSS/g COD. The highest VSS value was lower than the one referenced by other authors for membrane reactors (Cicek et al., 2001). Nevertheless, in previous experiments a negative effect was observed in the overall oxygen transfer coefficient by high suspended biomass concentrations in the reactor, so that the sludge concentration in the reactor could be a compromise in order to maintain enough biomass to achieve the removal of the contaminants without diminishing the oxygen capacity of the system.

The specific nitrifying activity of the suspended flocs was 0.22 g N-NH₄⁺/gVSS·d and its specific heterotrophic activity increased from 0.35 to 0.55 g O₂/VSS·d between the operating days 18 and 98. Sedimentation properties of the suspended biomass were poor, with a Sludge Volume Index (SVI) over 250 mL/gVSS. The bad settling properties did not affect the efficiency of the reactor as a result of the intrinsic characteristics of the system. Furthermore, the presence of suspended solid in the effluent was negligible as a result of the utilisation of the membrane module for biomass separation.

Information about the nitrifying and heterotrophic capacity of the unit is shown in Figures 4 and 5, respectively. Both, the total nitrifying and COD removal capacity of the system were calculated taking into account the specific activities and concentrations of the biofilms and the suspended biomass in the unit. Figure 4 shows information about the nitrifying capacity of the unit, the nitrifying capacity due to the presence of biofilms, and the percentage of the nitrifying capacity resulting from the presence of biofilms in the reactor.

The capacity of the unit increased progressively with time as a consequence of the increase of the biomass concentration of the flocs and biofilms. The capacity percentage, due to the presence of biofilms, increased from around 40 to 50% at the end of this stage.
The total nitrifying capacity of the unit, up to 2 kg N/m³·d, was around 250% higher than the applied ALR. With regard to the heterotrophic capacity, the biofilm had between 20 and 40% of the estimated capacity of the unit, of up to 6 kg COD/m³·d. Microscopic observation showed that the flocs presented an open structure, with the presence of filamentous microorganisms and a lower fraction of other microorganisms, such as protozoa and metazoa. Microscopic observation of the support showed that the biofilm’s growth occurred preferentially in the support valley, in which biomass was protected from the shear stress.

Nitriﬁng-denitriﬁcation stage (days 98–166)

From operating day 98 on, the anoxic chamber was coupled to the aerobic reactor in series, as shown in Figure 6, thus suspended biomass concentration diminished from 5.1 to 2.5 g SSV/L. Only during the first days of period 6 was there accumulation of ammonia in the effluent (Figure 6). After 20 operational days (day 118) the organic matter and ammonium removal were above 96%, as occurred during the previous period. The total nitrogen concentration and the total COD in the effluent were 68 mg/L and 114 mg/L, respectively.
Nitrogen removal percentage was 76%, which was very close to the theoretical value of 80% that is predicted with a recycle ratio of 4. It was not feasible to increase the recycle ratio and thus nitrogen efficiency as a consequence of the pump’s operational limits in the laboratory scale unit.

During period 6, OLR, ALR and nitrogen volumetric rates in the system were 5.2 kg COD/m³·d, 1.6 kg N-NH₄⁺/m³·d (referred to aerobic volume), and 1.24 kg N₂/m³·d (referred to anoxic volume), respectively, which were much higher than those attained in traditional activated sludge systems. The results obtained were similar or even better than those referenced for other predenitrification hybrid systems when these operated at similar OLR and ALR (Tanaka et al., 1991; Münch et al., 2000) and they were higher than those attained for membrane reactors which use suspended biomass (Ghyoot et al., 1999; Rosenberger et al., 2002). Theoretically, the recycling ratio necessary to reach nitrogen effluent concentration below the range between 10 and 50 mg/L (Spanish legal requirements for industrial wastewater) should be 32 and 6.4, respectively.

During period 7, OLR was halved in order to simulate the behaviour of the unit during the low activity periods of the factory, in which the wastewater production diminished. The reduction of the load (0.85 kg N-NH₄⁺/m³·d referred to aerobic volume and 2.5 kg COD/m³·d referred to total volume) and the increase of the recycling ratio to 5, made the reduction of the total nitrogen in the effluent at values below 10 mg/L feasible (nitrogen removal percentage above 80%). The effluent COD concentration was below 30 mg/L, the removal percentage being around 98%. The system during this period showed a high stability in the operation without accumulations of nitrogen or organic compounds.

During period 6 (days 98–143) the biofilm concentration increased until values above 3.3 g COD/L while the nitrifying activity increased from 0.68 to 0.78 g N-NH₄⁺/g protein·d and the heterotrophic activity from 2 to 3 g O₂/g protein·d; meanwhile the biofilm nitrifying and heterotrophic capacities increased gradually from 0.9 to 1.2 g N/L·d and from 2.8 to 4.1 g O₂/L·d, respectively. For suspended biomass the initial concentration was 2.5 g VSS/L, the final concentration being 5.1 g VSS/L, the specific heterotrophic, denitrifying and nitrifying activities achieved values up to 0.7 g O₂/g VSS·d, 0.22 g N-NO₃⁻/g VSS·d and 0.18 g N-NH₄⁺/g VSS·d, respectively. Total nitrifying and heterotrophic capacities of the system were 2.0 g N/L·d and 8 g O₂/L·d, respectively. Biofilms accounted for 70% and 55% of the total nitrifying and heterotrophic capacity of the system, respectively.

![Figure 6](https://iwaponline.com/wst/article-pdf/48/6/301/423568/301.pdf) Evolution of organic and nitrogenous compounds COD_total influent (■), COD_total effluent (□), total nitrogen in influent (⊗), total nitrogen in effluent (○)
During period 7 (days 144–166) the biofilm concentration decreased 10% and the specific heterotrophic activity 32%, however the nitrifying activity was practically constant. For the suspended biomass, the concentration was 5.5 g VSS/L and the nitrifying, denitrifying and heterotrophic specific activities diminished until 0.12 gN-NH₄⁺/gVSS·d, 0.18 g N-NO₃⁻/gVSS·d and 0.62 g O₂/gVSS·d, respectively. The appearance of the biomass under the microscope was similar to its appearance in the previous stage. The amount of sludge produced in membrane systems was low (0.2 g VSS/gCOD), thus the sludge purge was not so problematic as in conventional activated sludge systems. The predenitrification membrane hybrid system had an ammonium oxidation capacity (suspended and attached nitrifying activity) up to 2.0 k N-NH₄⁺/m³·d which was 250% that applied. On the other hand, the presence of suspended solids did not affect the specific capacity of the biofilm as occurred in the previous stage.

Conclusions
The combination of a hybrid system, both with suspended and attached biomass, and an ultrafiltration membrane module might be an alternative for treating wastewaters in a compact biological system. The intrinsic characteristics of the system made it feasible to operate the system at high OLR without the problems related with the poor settling properties of the sludge or the drop in nitrogen conversion. There were no solids in the effluent as result of the utilisation of the membrane ultrafiltration module. The membrane assisted hybrid reactor was used successfully to treat a mixture of two streams generated in a fish canning factory, one which had previously been treated in an anaerobic contact unit and had a significant COD and N content. Experiments were carried out during two periods: in a first experimental period, with aerobic conditions, it was feasible to achieve high ammonia and COD removals (98%) at high OR and NLR of 4.5 kg COD/m³·d and 1.8 kg N-NH₄⁺/m³·d, respectively. The biomass nitrifying activities were 0.68 g N-NH₄⁺/g protein·d and 0.16 g N-NH₄⁺/gVSS·d for attached and suspended biomass, respectively. During the second period, nitrification-denitrification period, results showed that it was possible to attain a maximum denitrification rate of 1.35 N-NO₃⁻/m³·d with nitrogen removal percentages of around 78%.

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References


