Nitrification of waste waters from fish-meal factories

Juan M Garrido, Lorna Guerrero, Ramón Méndez* and Juan M Lema
*Department of Chemical Engineering, University of Santiago de Compostela , Av. das Ciencias s/n,
Santiago de Compostela, E-15706, Spain*

Abstract

Fish-meal factories generate waste waters with high chemical oxygen demand (COD), suspended solids (SS), fats and protein concentrations. After their treatment by anaerobic digestion, effluents with high ammonia- and organic-nitrogen contents and a fraction of residual COD are produced. A biological nitrification/denitrification process is proposed to reduce both the residual carbon and the nitrogen. The aerobic stage has the double objective of reducing COD and nitrifying the ammonia- and organic-nitrogen for its further removal at the denitrification stage. The nitrification process of the waste water is presented here. Under operating conditions, ammonia accumulation in the aerobic reactor occurs, inhibiting nitrite-oxidising bacteria. Specific removal rates of COD (up to 1.5 g COD/g VSS·d) and TKN (up to 0.25 g N/g VSS·d) were obtained. The effect of nitrogen loading rate (NLR) on ammonia conversion was studied. A linear relationship was found between NLR and ammonia oxidation rate up to NLR of 0.6 g-TKN/ℓ·d, being the slope 0.57 g/g and the correlation factor of 0.96. With an NLR of higher than 0.6, it was not possible to obtain a nitrification rate higher than 0.5 g N-NO$_x^-$/ℓ·d. Percentages of COD removal ranged from 95% to 60% and the nitrification percentages from 65% to 20%.

Notation

COD   Chemical oxygen demand (mg/ℓ)
HRT   Hydraulic residence time
NLR   Nitrogen loading rate (g-TKN/ℓ·d)
OLR   Organic loading rate (g-COD/ℓ·d)
SS   Suspended solids
SRT   Solids retention time
TKN   Total Kjeldahl nitrogen (mg/ℓ)
TSS   Total suspended solids
VSS   Volatile suspended solids (mg/ℓ)
SVI   Sludge volume index

Introduction

Fish meal is produced either by using whole fish as raw material as is the case in most of the fish-meal factories of South America or from fish-canning factory residues as occurs in Europe. The waste water produced by fish-meal factories has high concentrations of SS, COD (especially due to the presence of fats and proteins) and nitrogen from proteins. Physical-chemical methods such as coagulation-flocculation or centrifugation can be used to recover solids (especially insoluble proteins) to be recycled to the fish-meal production (Mac Connis and Litchfield, 1989; Guerrero et al., 1993). An additional advantage is the reduction of COD in the effluents.

Once the solids are separated, most of the soluble COD of the waste waters can be efficiently removed by anaerobic digestion (Guerrero et al., 1993), as in the case of other waste waters with similar characteristics produced by the fish canning industry (Mac Connis and Litchfield, 1989; Veiga et al., 1994; Méndez et al., 1995; Omil et al., 1995). In spite of the high efficiency reached in anaerobic treatment, an organic load accounting for about 20% of the initial COD remains in the waste water, thereby necessitating a further treatment for COD and nitrogen removal.

In summary, the particular characteristics of these waste waters are their high nitrogen content as ammonia or organic nitrogen, their salinity and quite a high COD, which can complicate the nitrification step.

In order to lower the ammonia concentration, a stripping system can be used as a first step before nitrification/denitrification. Ammonia stripping is recommended for treating waste waters with ammonia concentrations of the order of g/ℓ whereas a biological denitrification/nitrification treatment is often required to fulfil the legal specifications on COD and nitrogen concentrations in the effluents.

Nitrification consists of the ammonia oxidation in two sequential steps to nitrite and nitrate respectively by *Nitrosomonas* and *Nitrobacter*. Biological denitrification is achieved under anaerobic conditions by heterotrophic bacteria using nitrate or nitrite as electron acceptors when a carbon source is available as an electron donor (Wiesmann, 1994). The sequential reactions involved in the nitrification/denitrification processes are described by the following equation:

$$NH_4^+ \longrightarrow NO_2^- \longrightarrow NO_3^- \longrightarrow NO_2^- \longrightarrow N_2 \qquad (1)$$
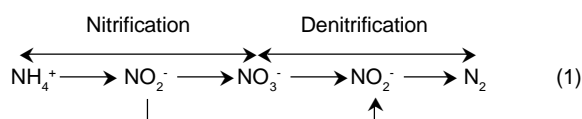
Nitrate is usually the final product of the nitrification process, although nitrite accumulation occurs if operational conditions, especially pH and ammonia concentration, are high enough to result in the inhibition of *Nitrobacter* by free ammonia (Suthersand and Ganczarczyk, 1986; Abeling and Seyfried, 1992).

Full removal of organic matter in the denitrification system is not achieved and part of the COD will be oxidised in the same systems in which nitrification occurs. The presence of organic matter in the aerobic system, which promotes the growth of heteretrophs, inhibits ammonia oxidation (Hanaki et al., 1990) and this precludes the feasibility of obtaining high ammonia conversions in the system.

In this paper we study the nitrification and removal of COD of the waste waters from fish-meal factories that were previously treated by anaerobic digestion.
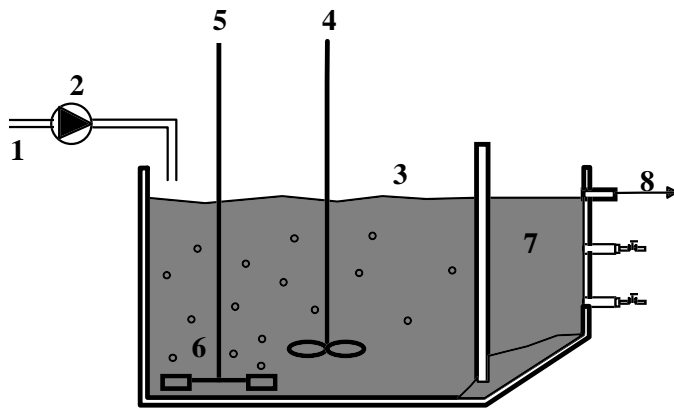
Figure 1
Laboratory activated sludge unit. 1 water inlet, 2 peristaltic pump, 3 aerated tank, 4 stirrer, 5 air inlet, 6 air diffuser, 7 settler, 8 water outlet

TABLE 1
WASTE-WATER CHARACTERISTICS

Parameter mg/ℓ	Synthetic water	Industrial waste water
COD	580	480 - 1 600
TKN	55	100 - 1 000
N-NH$_4^+$	25	100 - 850
Cl$^-$	-	< 5 000

TABLE 2
STRATEGY OF ADAPTATION AND OPERATION. 1 ADAPTATION, INFLUENT COMPOSED OF INDUSTRIAL WASTE WATER DILUTED WITH SYNTHETIC WATER. 2 OPERATION, INFLUENT COMPOSED OF INDUSTRIAL WASTE WATER DILUTED WITH TAP WATER

Operation time (d)	Industrial waste water (%)	COD (mg/ℓ)	TKN (mg/ℓ)
1 - Adaptation			
< 0	0	580	55
1 - 2	5	591	117
3 - 4	10	634	171
5 - 8	15	808	250
9 - 16	10	814/1245	189/267
2 - Operation			
17 - 20	15	579	287
21 - 23	20	503	366
24 - 27	30	1 189	486
28 - 30	43	789	873
31 -	60	1 630	997

Materials and methods

Analytical methods

TSS, VSS, pH and TKN were measured as proposed by *Standard Methods* (1985). The sludge volume index (SVI) was measured using a 50 mℓ graduate cylinder instead of a 1 ℓ as proposed by *Standard Methods* (1985). A semi-micro method, adapted for samples with a high chloride content (Soto et al., 1989) was used to determine the COD. Nitrate, nitrite, ammonia and oxygen were determined by using specific electrodes (*Standard Methods*, 1985).

All the parameters, including flow rate, were measured daily, except the SVI which was determined 3 times per week.

Laboratory-scale reactor

A laboratory-scale activated sludge unit of 1.7 ℓ working volume, built up in methacrylate, was employed as a treatment system (Fig. 1). A stirrer was used to guarantee sludge suspension. Aeration and additional mixing were provided by a membrane compressor. The settling compartment was separated from the aeration chamber by means of a baffle. The solid retention time (SRT) was controlled by purging sludge from the aeration compartment. Sludge recycling rate could be modified by displacement of the baffle located between the aeration chamber and the sedimentation tank. 0.3 ℓ of sludge from a municipal waste-water treatment plant was used as inoculum. Temperature was maintained at around 17 ± 0.8 ºC. Dissolved oxygen concentration in the reactor ranged from 1 to 4 mg/ℓ.

Waste-water characteristics

The unit was started up, some weeks before this study commenced, using a synthetic waste water with a similar composition to that of domestic waste water. Day 0 was considered as the first day when industrial waste water, stripped of anaerobic effluent, was used to feed the reactor (Table 1). In a period of 16 d the synthetic waste water was progressively replaced by the industrial effluent from the anaerobic treatment plant (Table 2). From day 16 onwards the unit was fed only with industrial waste water.

Results and discussion

After the start-up, the unit was operated at an SRT of 12 d whereas the HRT was varied from 0.89 to 0. 42 d. The pH of the influent ranged from 7.0 to 8.2, whereas the pH in the aerobic reactor was between 7.6 and 8.2. The VSS ranged from 600 to 2 300 mg/ℓ. SVI values of about 0.15 ℓ/g VSS were obtained once the system was fed with industrial waste water, this value indicating the good settling properties of the sludge. This is important because sludges with indices higher than 0.15 ℓ/g VSS frequently give rise to sludge carryover in the effluent from the final settling tank (Hänel, 1986). A high surface settler is therefore required in order to obtain proper solids removal.
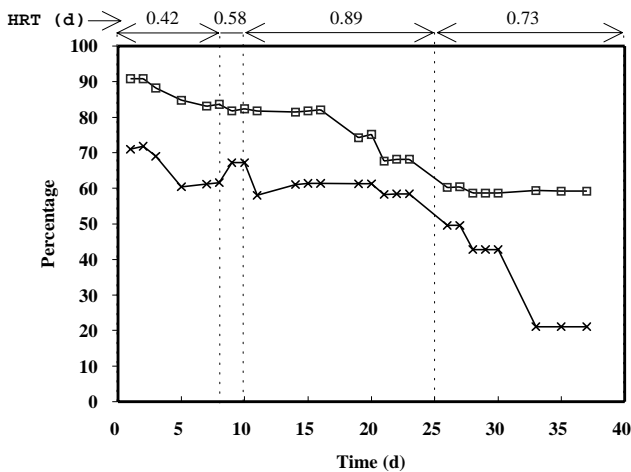
The CODs of the influent and effluent are presented in Fig. 2 and the efficiency of COD removal and nitrification can be observed in Fig. 3. Despite the use of a progressively higher percentage of
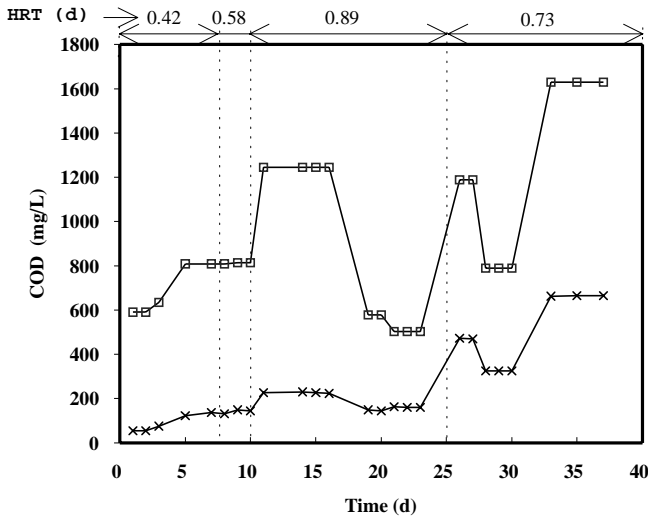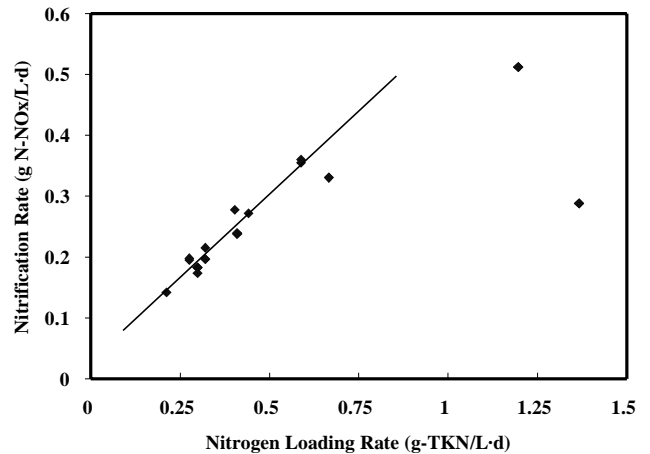
Figure 2
*Evolution of the COD in the aerobic reactor. (-□-) COD in
the influent (-x-) COD in the effluent*
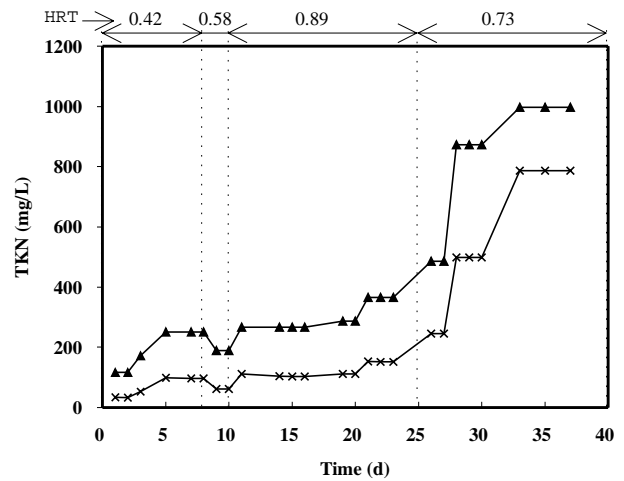
Figure 4
*Evolution of the TKN in the influent (−▲−) and
effluent (−x−)*

Figure 3
*Evolution of COD removal percentage (-□-) and
nitrification percentage (-x-)*

Figure 5
*Influence of NLR on nitrification rate in the reactor*

industrial waste water, drops in the COD in the influent were sometimes observed, due to the variability of the COD in the industrial waste water (Table 2). During the first operating days, COD removal was high, but it dropped when the percentage of industrial waste water was increased. It is interesting to note how, as a higher percentage of industrial waste water was used, a lower removal percentage was obtained. Moreover, once the adaptation period was completed, the COD removal was only about 60% which probably reflects the presence of non-biodegradable COD fraction in the influent that was not removed by either the anaerobic or aerobic treatment. This fraction accounts for about 10 % of the COD in the raw fish-meal waste water (average: 11 g COD/ℓ). The specific removal rate of COD (0.3 to 1.5 g COD/g VSS·d) and the volumetric removal rate of COD (0.4 to 1.6 g COD/ℓ·d) obtained were in the range of the expected values in activated sludge systems (Metcalf and Eddy, 1979).

The TKN values in the influent and effluent are shown in Fig. 4. The TKN in the influent was progressively increased from 0.15 g/ℓ to 1 g/ℓ. The fraction of TKN as ammonia changed from 78% in the influent to 95 % in the effluent, showing that almost the entire organic fraction of TKN in the aerobic reactor was hydrolysed to ammonia nitrogen. TKN of the effluent is lower due to the nitrogen requirement for biomass synthesis and the nitrification process. The percentage of nitrified TKN dropped from 60 to 20% when TKN and ammonia nitrogen concentrations increased in the influent, but the specific nitrification rate was higher (Table 3). As can be observed in Table 3, higher nitrification specific rates q_N (TKN converted to nitrite or nitrate nitrogen) were obtained working with industrial waste waters, when the COD/TKN ratio was lower (referred to previously) (Metcalf and Eddy, 1979). Variation of the volumetric nitrification rate obtained in the unit with the NLR is shown in Fig. 5. A linear relationship at lower NLR was observed. A value of 0.57 g/g and a correlation factor of 0.96 were obtained up to NLR of 0.6 g TKN/ℓ·d. With NLR higher than 0.6 it was not possible to obtain a nitrification

TABLE 3
NITRIFICATION SPECIFIC RATE EVOLUTION WITH THE COD/TKN RATIO

Operation time (d)	q_N g-N/g-VSS·d	COD/TKN ratio
3	0.10	3.7
20	0.21	2.0
35	0.25	1.7
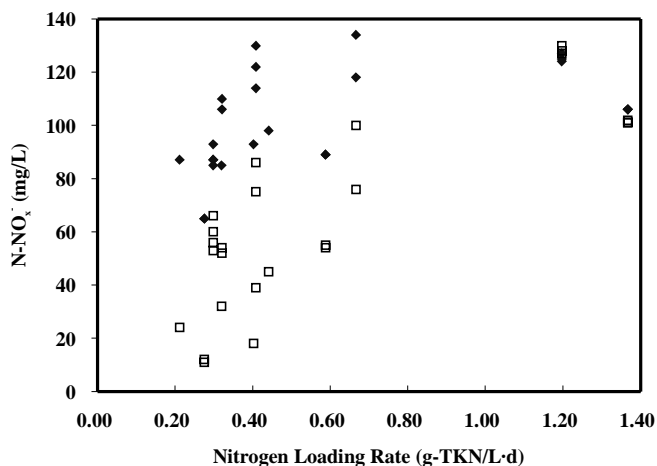
Figure 6
Evolution of the nitrite (□) and nitrate-nitrogen concentrations (♦) with the NLR in the reactor
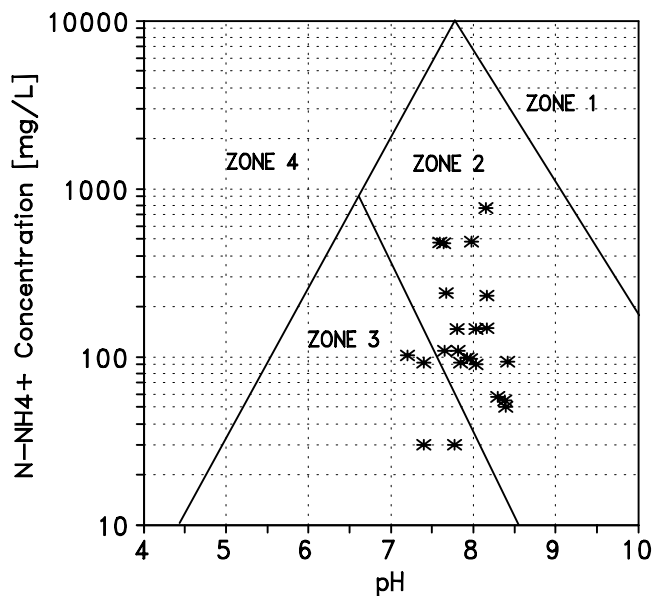
Figure 7
Working zone in the nitrification process

rate higher than 0.5 g N-NO$_x^-$/ℓ·d. Nitrification rates of between 0.2 and 0.5 g N-NO$_x^-$/ℓ·d were obtained during the experimentation period. These values are higher than those reported in the treatment of sewage in an activated sludge unit (0.2 g N-NO$_x^-$/ℓ·d).

The influence of NLR on nitrite- and nitrate-nitrogen in the effluent is presented in Fig. 6. NLR appears to have a stronger effect on nitrite than on nitrate concentration: The higher the NLR, the higher the nitrite concentration. Nitrogen-nitrite concentration reached 130 mg/ℓ when NLR was 1.2 g-TKN/ℓ·d. The effect of NLR on nitrate concentration is less pronounced and a positive effect is observed only when NLR was lower than 0.4 g-TKN/ℓ·d. At the beginning of this experiment, with lower NLR, 85 % of the oxidised nitrogen was converted to nitrate whereas, after the adaptation period, the percentage of nitrogen as nitrite increased progressively up to about 50%, when NLR was higher. In order to explain this fact, the experimental data were plotted in a chart proposed by Anthonisen et al. (1976) in which four zones are observed: Zone 1 - inhibition of *Nitrosomonas* and *Nitrobacter* by free ammonia; Zone 2 - inhibition of *Nitrobacter* by free ammonia; Zone 3 - complete nitrification; and Zone 4 - inhibition of *Nitrobacter* by free nitrous acid (Fig. 7). The operating conditions of the reactor during most of the experimental period were included in Zone 2, where a partial inhibition of *Nitrobacter* by free ammonia occurs and, accordingly, an important accumulation of nitrite is expected.

In an integral nitrogen removal process, nitrite accumulation could be positively influenced by the lower oxygen consumption in the aerobic stage (approximately 25%, energy savings) and the lower carbon source requirement in the anaerobic stage (approximately 40%). Despite these advantages, in this case, it is not feasible to operate the reactor with high ammonia concentrations as this suggests that low ammonia removal will be achieved in the system.

Oxygen is usually the limiting substrate for aerobic systems. Dissolved oxygen concentration was controlled between 1.5 and 4 mg/ℓ. In the reactor both ammonia and organic matter oxidising micro-organisms are competing for the oxygen. Heterotrophic micro-organisms compete favourably for the oxygen due to the lower growth rate and yield of nitrifying micro-organisms (Hanaki et al., 1990, Wiesmann, 1994). This fact limits ammonia oxidation as occurred in the aerobic system.

The main objective of this study was to demonstrate the feasibility of obtaining nitrification of the waste water from the fish-meal manufacturing process. High NLR provokes ammonia accumulation in the system, as the conversion is probably limited by the low presence of ammonia oxidisers in the sludge and nitrite accumulation due to *Nitrobacter* inhibition by free ammonia. In order to obtain a full ammonia oxidation of water with high ammonia and organic matter concentration applying high NLR and OLR, a second aerobic unit, treating the effluent of the first unit, might be used. In the first unit, oxidation of the organic matter would occur and in the second, the oxidation of ammonia (Wiesmann, 1994). If only one aerobic unit were used for the treatment of these waste waters and for obtaining a high or full ammonia conversion, it would be necessary to operate the system with lower NLR and OLR and thus operate it with higher HRT than those applied in our experiment.

The chloride concentrations, always lower than 5 g/ℓ, do not apparently affect the COD removal and the nitrification process is in accordance with previous results reported by Hänel (1986), Eilersen et al. (1994) and Gómez (1995). Specific nitrogen or COD removal rates were higher at the end of this experiment when the chloride concentration was higher.

Conclusions

Relatively high COD and TKN specific removal rates were obtained in this system (up to 1.5 g COD/g VSS·d and 0.25 g N/g VSS·d). The percentage of COD removal was limited by the presence of a non-biodegradable fraction in the waste water. Almost a complete hydrolysis of organic nitrogen to ammonia was obtained.

Nitrobacter was partially inhibited by free ammonia according to Anthonisen et al. (1976). A relatively high nitrite concentration was obtained, especially when operating the system at high NLR. Nitrite production in the aerobic system is interesting because lower oxygen consumption is required in the aerobic system and a lower electron donor and better denitrification rates might be obtained in an anaerobic denitrification treatment (lower chemical requirement and denitrification reactor volume). Despite these facts, it is not recommended to operate the system at high NLR as ammonia conversion under these conditions is not high.

Although ammonia removal was not high, several interesting facts were observed during this study:

- The COD removal percentage was 60% for the incoming waste water and up to 90% for the influent to the anaerobic digester.
- Almost all organic fractions of TKN were hydrolysed to ammonia in the aerobic process.
- Both nitrite and nitrate were produced and the ammonia oxidation was partly inhibited by the free ammonia concentration.

For these reasons we propose a biological nitrification/denitrification process with its aerobic stage in order to lower both COD and TKN for waste waters from fish-meal factories.

Acknowledgements

References

ABELING U and SEYFRIED CF (1992) Anaerobic-aerobic treatment of high strength ammonium wastewater nitrogen removal via nitrite. *Water Sci. Technol.* **26** (5-6) 1007-1015.

ANTHONISEN AC, LOEHR RC, PRAKASAM TBS and SRINATH EG (1976) Inhibition of nitrification by ammonia and nitrous acid. *J. Water Pollut. Contr. Fed.* **48** 835-852.

EILERSEN AM, HENZE M and KLOFT L (1994) Effect of volatile fatty acids and trimethylamine on nitrification in activated sludge. *Water Res.* **28** (6) 1329-1336.

GÓMEZ GJ (1995) The obtaining and stabilization of nitrifying sludge. In: *Proc. of the VI Natl. Congr. of Biotechnol. and Bioeng.* Ixtapa (Mexico), 10-14 September. 91 pp.

GUERRERO L, ALKALAY D, MÉNDEZ R and LEMA JM (1993) Treatment of fishmeal factory wastewaters. In*: Proc. of the 6th Eur. Congr. on Biotechnol.*, Firence-Italy, 13-17 June. pp THT262.

HANAKI K, WANTAWIN C and OHGAKI S (1990) Effects of heterotrophs on nitrification in a suspended-growth reactor. *Water Res.* **24** (3) 289-296.

HÄNEL K (1986) *Biological Treatment of Sewage by the Activated Sludge Process.* Ellis Horwood limited, London.

MAC CONNIS W and LITCHFIELD JH (1989) Meat, fish, and poultry processing wastes. *J. Water Pollut. Contr. Fed.* **61** (6) 855-858.

MÉNDEZ R, LEMA JM and SOTO M (1995) Treatment of sea food processing wastewaters in thermophilic and mesophilic filters. *Water Environ. Res.* **67** (1) 33-45.

METCALF and EDDY (INC.) (1979) *Wastewater Engineering: Treatment, Disposal, Reuse.* McGraw-Hill Book Company, New York, 776-788.

OMIL F, MÉNDEZ R and LEMA JM (1995) Anaerobic Treatment of sea food processing waste waters in an industrial pilot plant. *Water SA* **22** (2) 173-181.

SOTO M, VEIGA MC, MÉNDEZ R and LEMA JM (1989) Semi-micro COD determination method for high saline wastewater. *Environ. Technol. Letters* **10** 541-548.

STANDARD METHODS (1985) *Standard Methods for the Examination of Water and Wastewater.*(APHA-AWWA-WPCF) (16th edn.) Washington DC, USA.

SUTHERSAND S and GANCZARCZYK JJ (1986) Inhibition of nitrite oxidation during nitrification. Some observations. *Water Pollut. Res. J. Canada* **21** 257-266.

VEIGA MC, MÉNDEZ R and LEMA JM (1994) Wastewater treatment for fisheries operations. In: Martin AM (ed.) *Fisheries Processing: Biotechnological Applications.* Chapman and Hall, London.344-369.

WIESMANN U (1994) Biological nitrogen removal from wastewater. In: Fletcher A (ed.) *Advances in Biochemical Engineering.* Springer-Verlag, Berlin-Heidelberg. 113-154.