# Effect of the sludge recirculation rate on the performances of a two-stage anoxicsubmerged membrane bioreactor (A-SMBR) for the treatment of seafood wastewater

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#### Abstract

The objective of this research is to study the effect of the sludge recirculation rate on the performance of two-stage anoxic-submerged membrane bioreactor (ASMBR) for the treatment of seafood wastewater. The seafood wastewater presents the biodegradable of containing high values of organic matter (COD around 2750 mg.L<sup>-1</sup>) and organic nitrogen compounds (TKN around 484 mg.L<sup>-1</sup>). Four values of recirculation rates, from 1/8 to 3 times the inflow rate, were studied during a long operational period. The MBR reactor was operated in subcritical flux conditions and no sludge extraction was done during the whole experimental period. The overall efficiency for the COD and nitrogen compound removal rate was on average  $95\pm3\%$  and  $98\pm2\%$  respectively. The residual COD, TKN and NO<sub>3</sub><sup>-</sup>-N concentration in the effluent were  $23\pm4\_mg.L^{-1}$ ,  $1.2\pm0.9$  mg.L<sup>-1</sup> and  $29\pm9$  mg.L<sup>-1</sup> respectively. In terms of filtration, trans-membrane pressure (TMP) showed a small and constant values in the membrane reactor with a TMP value of around 5-10 mbars. Membrane fouling appeared linked with the soluble organic compound interactions on the membrane surface (irreversible fouling).The quality of treated water met with the standard of wastewater reuse for all classes (A to D) issued by USEPA.

Keywords: Denitrification, Membrane bioreactor, Nitrification, Sludge recirculation, Water reuse

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# 1. Introduction

Seafood industry represents a significant sector of Thailand's exports with an average annual income of 320 million dollars with an annual growth rate of approximately 10%. As the industry grows raw material requirements go up, water consumption demands become a key factor around 10-40 m<sup>3</sup> of water per ton<sup>-1</sup> of raw materials. Actually the existing wastewater treatment plants in Thailand do not have nutrients removal steps (which are conventional activated sludge and pond systems) and the treated water cannot be used for different reuse purposes. Due to the limitation of water sources and their degradation, the new wastewater treatment systems should be designed to favor on-site water reuse and should pass stringent discharge standards in the near future. In this context a global system, for both organic matter removal and nutrients compounds degradation should be made possible, as on-site water reuse seems indispensable for the seafood industry in Thailand. The wastewater generated from this industry contains a large part of easily bio-degradable food compounds as well as a high content of organic nitrogen.

A two stage anoxic-submerged membrane bioreactor system (ASMBR) is an attractive option for the treatment of such industrial wastewater. This configuration corresponds to a predenitrification configuration, anoxic reactor and aerobic MBR reactor, in which an external carbon source is not required [1-2]. Regardless of the environmental factors applied during denitrification step, free oxygen is a limiting parameter, which should be less than 0.2 mg/L. While oxygen compound in NO<sub>3</sub><sup>-</sup>N supplied from nitrification step is used for oxidizing organic matter in the influent. To provide the sufficient oxygen compound in NO<sub>3</sub><sup>-</sup>N and nitrogen removal by the denitrification step sludge age and sludge recirculation rate from nitrification tank (MBR) presents an important factor for the overall nitrogen removal in the system when using pre-denitrification and nitrification (pre DN). Its performances are well known in terms of organic matter and nitrogen removal efficiency, with the physio-chemical quality of the treated water below the stringent discharge standards. It is also favourable for water reuse onsite [3-6]. MBRs, especially submerged membrane bioreactors, are operated with high biomass concentrations, ranging from 8 to15 gMLVSS.L<sup>-1</sup>. This induces a low food-mass load (low F/M ratio) and is able to favour a reduction of sludge production [7-8].

Membrane biofouling is still of concern when optimizing the operating conditions in MBRs. The adsorption and pore blocking phenomena from macromolecules such as proteins, polysaccharides and organic colloid fractions, created from microbial metabolism, are still the major limitations on membrane permeability [3,7,9]. The subcritical flux operation has been widely used in wastewater applications as strategy to prevent and reduce membrane fouling [1-2,10] for long run operation periods. The objective of this work is to determine the effect of the operating conditions (the biomass recirculation rate) on the performance of a twostage anoxic submerged membrane bioreactor treating seafood processing wastewater.

#### 2. Materials and Methods

#### 2.1 Experimental set up

The experiments were carried out in a lab scale pilot (Fig.1). It was composed of an anoxic tank with a working volume of 14 L and an aerobic tank with a working volume of 24 L, where a microfiltration membrane module was installed. The operating conditions were performed to enhance both the biological process and membrane performance.

The mixing anoxic tank was conducted by the agitator which was controlled by a timer in order to achieve cyclic working of 30 minutes and a pause of 60 minutes. The evolution of the trans-membrane pressure (TMP) was monitored and measured by a negative pressure gage, which was connected directly to the membrane module on the permeate side. The pH was controlled at 7.0 - 8.0 by the addition of sodium bicarbonate (NaHCO<sub>3</sub>) and the temperature was maintained at room temperature.



Fig. 1. Schematic diagram of the experimental set up: (1) =Feed tank equipped with mixer, (2) = Anoxic tank equipped with mixer, (3) = Aerobic tank within hollow fiber membrane and equipped with pressure regulator and (4) Permeate tank

# 2.2 Membranes

The membrane module of anoxic-submerged membrane bioreactor, equipped with hollow fibers membrane module, was made of Polyethylene with a pore size of 0.22  $\mu$ m and effective filtration area of 0.2 m<sup>2</sup> (initial membrane permeability around 3000 L.h<sup>-1</sup>.m<sup>-2</sup>.bar<sup>-1</sup>). The airflow rate was fixed at 5 L.min<sup>-1</sup> for providing oxygen to favor the aerobic biological process (DO = 4-5 mg.L<sup>-1</sup>) and induced shear stresses around the membrane modules to minimize reversible phenomena causing membrane fouling. A critical value of 0.25 bars was set-up for the TMP and the characteristics of fouling were quantified after this critical TMP value reached. A specific cleaning procedure was practiced to determine resistance values at the end of operation. A cleaning was operated step by step as follow: (1) rinsing the membrane with only tap water until no sludge accumulation, (2) back washing with citric acid 1 wt.% at a low rate of 15 L.h<sup>-1</sup>.m<sup>-2</sup> for 60 minutes and (3) immersion in sodium hydroxide solution 1 wt.% for 120 minutes. The permeability of membrane was measured after each step of cleaning and quantifies the type and level of fouling occurred.

#### 2.3 Membrane fouling investigation

The fouling potential of sludge floc suspension from anoxic and MBR tanks was investigated. The filterability was examined and evaluated in frontal filtration mode to study the influence of mixed liquor suspended solids concentration and composition from anoxic and MBR reactors. The experimental set-up was a lab scale filtration unit with the selected commercial plane organic membrane as a common application of material type in several researches reported for predicting hydraulic resistance in membrane fouling [9, 11-12].

#### Table 1 Membrane characteristics

	VMWP 04700 Millipore	
Туре	Plane	Plane
Membrane material	Mixed cellulose ester	Nitrocellulose
Dimension	47	47
(mm, diameter)		
Filtration area (cm <sup>2</sup> )	11.9	11.3
Pore size (µm)	0.22	0.05
Porosity (%)	75	72
Thickness (µm)	180	105
Water flux (20 <sup>°</sup> C, 1 bar)	10800	400
$(L^{-1}.h^{-1}.m^2)$		
Membrane resistance	$2.5 \times 10^{6}$	$0.9 x 10^{12}$
$(R_m, m^{-1})$		

The unit consisted of a pressurized filtration cell with a working volume of 150 ml. and the characteristics of each membrane used are given in Table 1. Cake filtration theory was used to examine and explain the effect of the various components in suspension tested. The methodology consisted of following up the cumulated volume of filtrate during filtration time for given trans-membrane pressures (TMP) at 0.25 and 0.5 bar without any applied turbulence. The changes in the filtered volume can be described by the cake filtration law and the specific cake resistance can be determined.

#### 2.4 Feed wastewater, cultures and experimental runs

Real seafood processing wastewater (Surimi products) was used as feed suspension. It contains a large part of easily bio-degradable compounds, as well as a high content of organic nitrogen (pH 5.67). The average feed concentrations are given in Table 2. The wastewater was diluted with tap water in order to adjust the organic loading inlet. The diluted wastewater with an approximately constant COD of 700-1000 mg.L<sup>-1</sup>was continuously fed under a flow rate of 48 L.d<sup>-1</sup> into the anoxic tank. Aerobic mixed cultures of heterotrophic organisms from the existing activated sludge system in the seafood processing industry were used to inoculate the bioreactors.

 Table 2
 Mean concentrations of the original seafood

 wastewater

Parameters	<b>Mean concentrations</b> (mg.L <sup>-1</sup> )
COD <sub>Total</sub>	2750
BOD <sub>5</sub>	1500
TSS	150
TKN	484
Protein	156
Salinity	1000-2000

 Table 3 Summary of the operating conditions in a two-stage

 ASMBR system

Operating condition	Anoxic	MBR
	reactor	reactor
Working volume (L)	14	24
Hydraulic flow rate $(L.d^{-1})$	48	48(1+R)
Volumetric organic load (g $_{COD}$ .L <sup>-1</sup> .d <sup>-1</sup> )	2.4-3.4	1.4-2.0
Hydraulic retention time (HRT, d)	0.29	0.5
Sludge retention time (SRT, d)	> 100	
Temperature ( <sup>0</sup> C)	26-28	
pH	7	-8
Dissolved oxygen $(mg.L^{-1})$	<u>≤</u> 0.5	4-5

The system worked without sludge extraction with the objective of favoring high sludge age and minimizing sludge production. The sludge recirculation flow, from the MBR reactor to the anoxic reactor, was varied during the system operation. The recirculation factor (R) imposed during the four operational periods were: 1/8, 1/2, 1 and 3 of the inflow rate in the anoxic reactor. The critical flux was determined according to the flux step method [13] and a subcritical flux at 75% of critical flux was chosen. The experiments were operated at a low F/M ratio of 0.48 and 0.24 d<sup>-1</sup> in anoxic and MBR tanks. The systems were operated under the operational conditions (Table 3).

#### 2.5 Analysis

The performance of the bioreactors was studied by monitoring substrate degradation and biomass concentration evolution over time. Several parameters were determined in the influent, the effluent from the anoxic reactor, permeate and the mixed liquor suspensions, such as temperature, pH, DO, turbidity, color, MLSS-MLVSS, COD, BOD<sub>5</sub>, TKN, NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N by the standard methods of APHA et al., 1998 [14].

## 3. Results and Discussion

#### 3.1 Overall performances

# 3.1.1 Performances of organic matter (COD) removal

Figure 2 shows the evolutions of the total COD in the influent  $(COD_{TI})$ , the soluble COD in the influent  $(COD_{SI})$  and the effluent  $(COD_{TE})$  during the overall operational period. For the total and soluble influent COD average values equals to 851 and 264 mg.L<sup>-1</sup> were respectively observed. From the beginning of the operation an excellent quality of effluent was obtained, with a permeate COD

concentration of 29, 19, 23 and 21 mg.L<sup>-1</sup> respectively for each operational condition. The average COD removal efficiency was around 95±3% in the overall operational period and the turbidity of the effluent permeate was lower than 2 NTU.

Table 4 presents the average COD removal efficiencies at steady state condition for each operational period. It is interesting to note that in the first operational period the higher COD fraction was removed in the MBR system and not in the anoxic reactor.



**Fig. 2.**  $\text{COD}_{TI}(\bullet)$ ,  $\text{COD}_{SI}(\blacktriangle)$ ,  $\text{COD}_{TE}(\blacksquare)$  evolution and  $\text{COD}_{T}$  removal efficiency (**O**) during the anoxic-MBR system operation.

Conditions	Anoxic	Anoxic MBR	
	reactor	reactor	
Period R1/8	-	95	97 <u>+</u> 1
Period R1/2	56	75	98 <u>+</u> 1
Period R1	74	61	90 <u>+</u> 1
Period R3	88	49	93 <u>+</u> 1
Over all	54	70	95 <u>+</u> 1

 Table 4 Total COD removal efficiencies (%) in steady state

 condition for the two-stage ASMBR system

However, in the subsequent periods, the increasing of COD removal efficiencies was observed in the anoxic reactor. Probably the low COD removal efficiency observed in the anoxic reactor in the first period was linked to a pH not optimum, around 5.0-6.0, for the microbial activity. The pH was regulated in the subsequent periods.

# 3.1.2 Performances of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>N removal

Figure 3 shows the nitrogen compounds evolution in the influent and effluent. From the decreasing evolution of  $NH_4^+$ -N the progressive increase of the nitrification activity in the first operational period can be clearly observed. In the subsequent periods the  $NH_4^+$ -N concentration in the effluent was around 1 mg.L<sup>-1</sup>. That shows the excellent nitrification activity of the biomass in the aerobic MBR reactor. While the low nitrification activity in the first operational (77 %)

period was influenced by the pH not optimum for microbial activity. Both factors from the nature of the substrate variation and its dynamic and pH, decreasing due to the nitrification reaction, could be considered in this low pH occurrence. However, the pH control in the subsequent periods favored an increase in nitrification activity.

The evolution of the  $NO_3$ -N concentration showed an inverse tendency. In the first operational period, an increase in the  $NO_3$ -N concentration was observed, in accordance with the increasing of nitrification activity. After that, in

each subsequent operational period, a decreased evolution in the NO<sub>3</sub><sup>-</sup>-N concentration was gradually observed. This is in accordance with a good denitrification efficiency rates (Table 5) observed in the anoxic bioreactor when operated at recirculation factor (R) 1/8 and 1/2 and the dilution effect of the higher recirculation rate values. Nitrogen removal rate was completely 100% at recirculation factor (R) 1/2. In the last operation the NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>N concentrations in the effluent were 0 and 15.2 mg.L<sup>-1</sup> respectively.



Fig. 3. Evolution of the nitrogen compounds in the overall operational period: ( $\blacklozenge$ ) NH<sub>4</sub><sup>+</sup>-N influent, ( $\blacksquare$ ) NH<sub>4</sub><sup>+</sup>-N effluent and ( $\Delta$ )NO<sub>3</sub>-N effluent.

Conditions Anoxic reactor MBR reactor An			
Conditions	(% denitrification)	(% nitrification)	MBR
Period R1/8	100	77	89
Period R1/2	100	97	100
Period R1	85	97	70
Period R3	61	85	31
Over all	89	87	73

 Table 5 Percentages of nitrogen compounds removal

 efficiency in steady state conditions

The slightly decreasing of denitrification activity was observed in the anoxic reactor. This linked to the increasing of dissolved oxygen when higher sludge recirculation rate was operated in the anoxic reactor. The oxygen concentration varied in range from 0.5-1.4 mgO<sub>2</sub>.L<sup>-1</sup>. This aerobic condition inhibited the denitrification in anoxic reactor [15].

# 3.2 Biomass evolution and production

# 3.2.1 Evolution of Mixed Liquor Suspended Solids (MLSS) in the system

Figure 4 (a-b) shows the evolution of MLSS and MLVSS concentrations for the anoxic and MBR reactors respectively. The bioreactors were inoculated with the same sludge with a MLSS concentration equal to 5000 mg.L<sup>-1</sup>. In the anoxic reactor a slightly fluctuations in the concentration of MLSS and MLVSS were observed during the period of operation. In the MBR reactor a slightly increase evolution in both MLSS and MLVSS concentrations was observed after the first operational period. This was due to the effect of mixing inside the reactors.

Table 6 presents the values of the biomass production rate, the substrate consumption rate and sludge conversion yields. In the overall operational period the observed sludge yields were equal to 0.01 g VSS.g  $\text{COD}^{-1}_{\text{removed}}$  and 0.003 g VSS.g  $\text{COD}^{-1}_{\text{removed}}$  corresponding to 0.02 and 0.04 g $\text{COD}_{p}$ . g $\text{COD}_{T}^{-1}$  (using a factor of 1.42 g  $\text{COD}_{p}$ . g VSS<sup>-1</sup> [16]) for the anoxic and MBR reactors respectively. These values are approximately 25-50 times lower than those measured in a conventional activated sludge process [17]. The results point out clearly the lower sludge productions of the two-stage Anoxic-MBR system. The low biomass productions obtained in both reactors showed that the substrate was principally not allocated to the cell growth functions. 
 Table 6 Biomass production rate, substrate consumption rate

 and sludge yields

	Anoxic	MBR
	reactor	reactor
Biomass production rate	0.007	0.002
$(gCOD_p.L^{-1}.d^{-1})$		
Substrate consumption rate	0.43	0.46
$(gCOD_T L^{-1} d^{-1})$		
Biomass production rate/substrate	0.02	0.004
consumption rate		
$(gCOD_{p}, gCOD_{T}^{-1})$		
Observed sludge yield	0.01	0.003
(g VSS.g COD <sup>-1</sup> <sub>removed</sub> )		



(a) Anoxic



(b) MBR

Fig. 4. MLSS and MLVSS production in Anoxic-MBR

In substrate/biomass limited conditions (low F/M ratios); literature reports that the substrate would be essentially consumed in order to ensure the cell maintenance requirements instead of growth functions [18-20]. Both reactors were operated at a low F/M ratio close to 0.24 d<sup>-1</sup>. The evolution of the ratio MLVSS/MLSS in the microbial suspension showed a slightly accumulation of inorganic substances. A nearly constant value of around 0.8 was observed for both anoxic and MBR suspensions, in the overall operational period. However, it was observed that in the first and second periods had a slightly decreased evolution from 0.8 to 0.78, probably due to inorganic matter accumulation coming from the influent.

Mixed liquor suspension analysis was performed to investigate the morphology of floc and microbial compositions (Fig. 5) that may be affected by some variation of substrate characteristics such as salt concentration between 2000-3000 mg.L<sup>-1</sup> and COD loading with low F/M close to 0.24 d<sup>-1</sup> in MBR under real conditions of wastewater inlet. It appeared slightly loosen floc type with free swimming ciliates, which was higher than fixed ciliates and rotifer at the beginning period. Floc density increased with time as some of filamentous bacteria presented in microstructure of floc. The composition of microbial presented in MBR and their characteristics not

only showed healthy floc formations and good settling but also confirmed the system did not affect from sludge recirculation rate after 15 days without overgrowth filamentous bacteria occurred in macrostructure of floc. This also was not causing membrane fouling. Surveying of microbial compositions in each experiment confirmed the system stability with a good quality of permeate according to the presence of protozoa such as *sarcodia fagellata* and cilita in the beginning, stalked ciliates after 60 days (b-1-2), *suctoria* and free swimming ciliates after 90 days (c-1-2) and rotifers in MBR tank after 120 days. This result conveys the tolerance characteristics of microbial towards salt concentrations in the conditions applied.



**Fig. 5.** Floc morphology investigation and Group of metazoan: (a) Seed floc at start up period with free swimming Cilia (x100), (b-1) (x10)-in anoxic tank and (b-2) (x10)-in MBR tank of sludge recirculation 1/2 after 60 days, (c-1) (x20)-in anoxic tank and (c-2) (x10)-in MBR tank of sludge recirculation 1 after 90 days, (d-1) (x20)-in anoxic tank and (d-2) (x10)-in MBR tank of sludge recirculation 1 after 120 days

# 3.2.2 Filterability of sludge floc suspension

Figure 6 and Table 7 present the evolutions of t/V vs. V and  $\alpha$ .W values obtained for different operating conditions tested on filterability of sludge floc suspension. Three different filtration experiments, filtration of the raw suspension (particular and soluble fractions) from anoxic

reactor and MBR reactor and filtration of the only soluble fraction, have been studied with the objective to show the influence of the composition and concentration of the biological suspension. Figure 6 shows that the values of  $\alpha$ .W increased with concentration of raw suspension and pressure applied at the same condition tested.



# (a) MLSS from anoxic reactor



(b) MLSS from MBR reactor

Fig. 6. Evolution of filterability (t/V VS V) of raw suspension: (a) MLSS from anoxic reactor and (b) MLSS from MBR reactor

MLSS	$\alpha.W(10^{12},m^{-2})$			
$(mg.L^{-1})$	TMP = 0.5 bar		TMP = 0.25  bar	
	0.22 μm	0.05 μm	0.22 μm	0.05 µm
Anoxic-3000	99	124	71	71
Anoxic-1500	57	99	28	71
MBR-3000	71	99	50	71
MBR-1500	43	85	21	64
Soluble fraction				
Anoxic-1500	not analyzed	28	not analyzed	not analyzed
MBR-1500	not analyzed	28	not analyzed	not analyzed

Table 7 (C.W values obtained from dead end filtration of MLSS tested

When filtering raw suspension with 0.05  $\mu$ m the values of  $\alpha$ .W were higher than the values obtained from 0.22  $\mu$ m. This was because high external fouling occurred since most of particular fraction could be retained totally on membrane surface faster than soluble fractions could form or penetrate. The results show that the values of  $\alpha$ .W from filtration of raw suspension from anoxic reactor were a little bit higher than the values obtained when filtration of raw suspension from MBR reactor. However, the values of  $\alpha$ .W when filtering soluble fraction were quite lower 3-4 times than filtering raw bacterial suspension (MLSS = 3000 and 1500 mg.L<sup>-1</sup>) when the filtration was done with 0.05  $\mu$ m membrane for 0.5 bar pressure applied.

This was because the filtering of soluble compounds, which had very low residual concentration of organic matter even with smaller pore sizes of membrane, allowed compounds that had random structures over membrane pore and most of them may be penetrate due to their molecular weight was too small comparing with membrane pore size. In addition, they could be adsorbed in pore channel so that thin layer of cake and/or jelly formation occurred on membrane surface.

#### 3.3 Fouling in the MBR reactor

Due to the totality of the suspended solids and biomass retention in the system, the evolution of TMP during operation was monitored and it was found that there was no increase of the TMP in the Anoxic-MBR system even at high MLSS concentrations. The TMP value was nearly constant with a very low value of between 5-10 mbars. In addition, the hydrodynamic conditions and the operation at low permeate flux in a subcritical condition avoided major fouling and allowed no significant rapid reversible fouling from the biomass cake layer on the membrane surface [21] Please remove from the text the hydraulic resistance of the membrane (R) is widely recognized as an important parameter to monitor the filtration performances. Figure 7 shows the resistance of the membrane measured after each cleaning step by filtering water. This measure was done at the end of the two-stage ASMBR system operation to identify and quantify the type of membrane fouling (see topic 2.2).

The quantification of different resistance values shows that the reversible fouling (due to biomass accumulation on the membranes) is negligible.



**Fig. 7.** Resistance of the membrane measured after each cleaning step by filtering water (Rt = total resistance at the end of filtration, Rm = resistance of clean membrane, Rre = reversible resistance and Rirre = irreversible resistance after chemical cleaning)

However, after the chemical cleaning of the membranes, an 84 % of the initial membrane permeability was recovered. Therefore, the irreversible fouling for soluble compounds interaction (notably proteins, polysaccharides and humic substances) with the membrane surface is the critical factor in membrane fouling.

#### 4. Conclusions

Biological and filtration performances were investigated in a two-stage Anoxic-MBR using real seafood wastewater. The effect of the sludge recirculation rate on the performances of the system (organic and nitrogen compounds removal) was studied from R1/8 to R3 of the inflow rate. The results showed a high COD degradation and nitrogen compounds removal, with global efficiencies in the last operational period of 95+3% and 98+2% respectively. The dilution effect of the high recirculation rates allowed lower NO<sub>3</sub>-N concentration in the effluent even if the denitrification efficiency in the anoxic reactor was decreased. The quality of treated water is in accordance with the Thailand discharge industry standards and also the standard of wastewater reuse for all classes (A to D) issued by USEPA. The filtration conditions allowed a long operational period without the requirement of membrane regeneration. Considering the biological and filtration performances in the two-stage Anoxic-MBR appears as a sustainable system for the treatment and reuse of wastewater coming from the seafood industry.

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