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DECREASED SLUDGE PRODUCTION STRATEGY FOR DOMESTIC WASTEWATER TREATMENT

Angel Canales*, Alain Pareilleux*, Jean Luc Rols*, Gérard Goma* and Alain Huyard**

** Département de Génie Biochimique et Alimentaire, URA-CNRS-544, Institut National des Sciences Appliquées (INSA), Complexe Scientifique de Rangueil, 31077 Toulouse, France*
*** Centre International de Recherche Sur l'Eau et l'Environnement (CIRSEE), Lyonnaise des Eaux-Dumez, 38 Avenue du Président Wilson, 78230 Le Pecq, France*

ABSTRACT

With new EEC regulations, alternative treatment and disposal techniques of the excess sludge produced by Activated Sludge (AS) wastewater treatment plants have to be performed. In order to reduce the excess sludge produced, experiments have been carried out with a Membrane BioReactor (MBR) to study the maintenance and cryptic growth phenomena of *Pseudomonas fluorescens* culture taken as a model when grown on a limiting substrate complex medium similar to a synthetic urban wastewater. Experiments with various imposed wasting rates showed that viability and sludge production yield decreased when sludge age increased. Same variations were observed on the cell content ratio protein/polysaccharide by analysis of the cell lysis products released after discontinuous thermal treatment. Biomass growth on these cell lysis products was achieved to characterize cryptic growth and its impact on sludge production yield. Finally, a continuous sludge thermal treatment system was operating with MBR to amplify sludge breakage and consequently biomass growth on the lysis products. With the promising results obtained, this work gives a new outlook on the AS process and leads to the development of processes with control and reduction of sludge production.

KEYWORDS

Wastewater treatment ; activated sludge ; membrane bioreactor ; sludge production ; cell lysis ; cryptic growth.

INTRODUCTION

Conventional (activated sludge) processes for wastewater treatment transform for organic pollution into gas (CO_2 or CH_4), water biomass. The cost of the excess sludge treatment and disposal can represent up to 60% of the total operating costs. Therefore, the AS process transfers a water pollution control problem into a solid waste disposal problem. According to the new EEC regulations, the sludge disposal to agriculture or landfill becomes difficult. In large wastewater treatment plants, aerobic or anaerobic sludge digestion processes are used to reduce this excess sludge production.

In the AS process, different parameters influence the sludge production : the sludge age, the substrate limitation, etc... To find limiting steps for the reduction of excess sludge production and to avoid the constraint of a settling tank in an AS process, an MBR treating a synthetic wastewater was used to investigate the different physiological activities of the biomass (death, lysis, growth on intracellular products, etc...). The MBR, a combination of a biological reactor with membrane separation techniques, presents the

advantage of a complete dissociation and control of the hydraulic and biomass retention times. With the perfect control of these two process parameters, the MBR can work with high biomass concentration, decreasing the ratio of the food/microorganism concentrations and amplifying the maintenance phenomena (Bouillot *et al.*, 1990 ; Chaize and Huyard, 1991). Introduced by Pirt (1965), this concept describes the reduction of the substrate/biomass conversion yield observed with bacterial population under limiting substrate conditions. The maintenance concept resumes different bacterial reactions like death, lysis, endogenous metabolism and is important in wastewater treatment (Jones, 1973).

Using carbon limited cultures of *Klebsiella pneumoniae*, Mason and Hamer (1987) have studied the viability of the cells and phenomena of cell death, lysis and growth on cellular products released (cryptic growth). According to these authors, the biodegradation of the cell wall is a rate-limiting step and to increase it physical or chemical treatments are used in dowstream processing (Harrison, 1991). Addition of a thermal pre-treatment improves the sludge digestibility (Haug, 1978 ; Hiroaka *et al.*, 1985).

The aim of this paper is to study the viability of the biomass and the phenomena associated with the maintenance concept. To increase the death and the lysis of the biomass, a thermal treatment was added in line on a recirculation loop on the MBR. Variations of the maintenance coefficient of the biomass on the MBR, fed with soluble or total lysis products, were measured.

MATERIAL AND METHODS

Strain and growth medium

Pseudomonas fluorescens ATCC 13525 was used to simulate the behaviour of mixed cultures in an AS process. This microorganism is commonly present in soil, water and AS.

The growth medium contained organic fractions ($mg.l^{-1}$) : acetate, 400 ; sucrose, 400 ; peptone, 150 ; yeast extract, 50 ; and salts ($mg.l^{-1}$) : $(NH_4)_2HPO_4$, 200 ; $(NH_4)_2SO_4$, 200 ; K_2SO_4, 30 ; $MgSO_4$-$7H_2O$, 30 ; $FeSO_4$-$7H_2O$, 5 ; $CaCl_2$, 5. The organic substrates were chosen to simulate domestic wastewater with a concentration of 1 $gCOD.l^{-1}$.

Laboratory pilot plant

The pilot plant, described previously by Bouillot *et al.* (1990), was composed of a completely mixed biological reactor (volume 6 litres) associated with an ultrafiltration module loop (mineral membranes, Carbosep-SFEC). Membrane cut-off was around 200,000 daltons and the module surface area was $0.16m^2$. This pilot plant can be used with different configurations which are presented in figure 1.

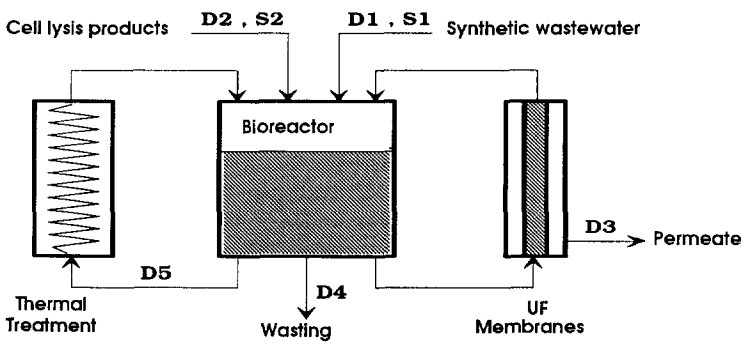

Fig. 1. Configurations of the laboratory pilot plant.

The substrate can be the synthetic wastewater S_1 fed at dilution rate D_1 and/or the cell lysis products S_2 fed at D_2. The permeate of membrane module was extracted at dilution rate D_3. Finally, a wasting rate can be imposed on the MBR through D_4 or D_5 when thermal treatment was operating. With this pilot, a complete dissociation of the hydraulic and the biomass retention times enabled to fix the specific growth rate of the culture by adapting the wasting rates D_4 or D_5. The different pilot configurations used were :

1) MBR fed with synthetic wastewater (D_2=0 and D_5=0),
2) MBR fed with synthetic wastewater and cell lysis products (D_5=0),
3) MBR associated with sludge thermal treatment (D_2=0 and D_4=0).

The operating conditions were the following : temperature, 29°C ; pH, 7.5 (regulated by NaOH supply) ; dissolved oxygen concentration , 30% of the air saturation.

In some experiments, cell lysis was induced by physical treatments (mechanical or thermal) on the sludge extracted from the bioreactor through the wasting rates D_4 or D_5.
For cell lysis by mechanical treatment, excess sludge from the MBR was centrifugated at 20,000 rpm for 10 minutes and washed with fresh water. Then the biomass was ground with a Dino-Mill type KDL device using glass beads (diameter 0.25 mm) d for g 10 minutes. The raw lysis products or the filtered products on the membrane (0.2µm) were injected in the bioreactor with the hydraulic dilution rate D_2.
For cell lysis by thermal treatment, sludge was heated at 90°C for 3 hours and then recycled in the bioreactor with the wasting rate D_5.

Analytical methods

The Total Organic Carbon (TOC) was determined using an infrared analyser "Ionics Model 1258" and the Chemical Oxygen Demand (COD) was calculated with COD = TOC x 2.65. Biomass concentration was expressed in dry weight obtained by membrane filtration (Millipore AP 20) and drying 24 hours at 110°C. Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951) and polysaccharide concentration by the Anthrone method (Dreywood, 1946). The measurement of biomass viability was performed by the INT method (dehydrogenasic activity test) from Lopez *et al.* (1986), by the differential centrifugation method (described previously by Bouillot, 1988 ; and Canales, 1991), or by the enumeration method on Petri dishes.

RESULTS AND DISCUSSION

Viability and sludge production yield in MBR

Because the hydraulic dilution rate D_s and the wasting rate D_b can be controlled separately, the MBR is suitable to study the different physiological states of biomass depending on sludge age. The most important physiological state to focus on is the viable biomass defined as the biomass able to consume substrate for its own growth. The substrate removal rate and the sludge production yield are dependent on the level of cell viability. In a first experiment, the MBR (see figure 1 with D_2=0 and D_5=0) was fed with synthetic wastewater (S_1=1 gCOD.l^{-1}) with three dilution rates (D_1=0.5, 0.8 and 1.1 h^{-1}) combined with various wasting rates (D_4=0.2, 0.1, 0.04, 0.025, 0.015 and 0.0075 h^{-1}). The results, obtained at steady states, including biomass viability and sludge production yield $R_{X/S}$, are presented respectively in figures 2 and 3.

Concerning the viability of *P. fluorescens* (figure 2) determined by two different analytical techniques (differential centrifugation and INT test), it was shown that the greater the wasting rate (or the smaller was the sludge age), the greater was the biomass viability. The biomass viability was above 95% when the biomass residence time was under 10 hours. The comparison of these results with those of Postgate and Hunter (1962), for *Aerobacter aerogenes* growing in chemostat, shows that the relationships between the percentage of viable biomass and the net growth rate are similar for different methods of viability

measurement. With ATP activity measurement method, Weddle and Jenkins (1971) have found a cell viability of 15% in an AS process under low net growth rate (0.026 to 0.3 d^{-1}). Moreover, the biomass viability was shown to be independent of the hydraulic residence time. This observation is in agreement with results obtained by Lafforgue (1988) who was working with total recycling of *Saccharomyces cerevisae* cells in MBR.

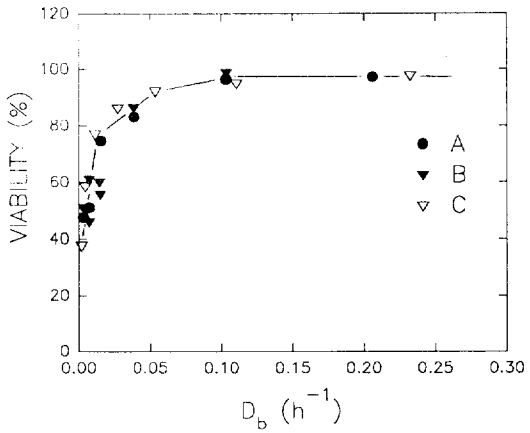

Fig. 2. Influence of the wasting rate on the biomass viability for *P. fluorescens* cultures measured : (A) by the INT method, and (B) by the differential centrifugation method, and for *A. aerogenes* cultures (C) measured by the enumeration method (from Postgate and Hunter, 1962).

Concerning the sludge production yield of *P. fluorescens* (figure 3), it was shown that the greater the wasting rate, the greater was the sludge production yield. Biomass with low sludge age had a high viability level and consequently used COD for growth. In this condition, the corresponding sludge production yield was very high and stretched to a limit $Y_{X/S}$=0.57 gbiomass.g^{-1}COD (Bouillot, 1988). In contrast, biomass with high sludge age had a low viability level and COD removal associated with requirements for maintenance was higher than for growth. A maintenance coefficient of 0.035 gCOD.g^{-1}biomass.h^{-1} was calculated. Verstraete and Voets (1978) obtained the same value with *P. fluorescens* cells growing in a chemostat under glucose limitation conditions.

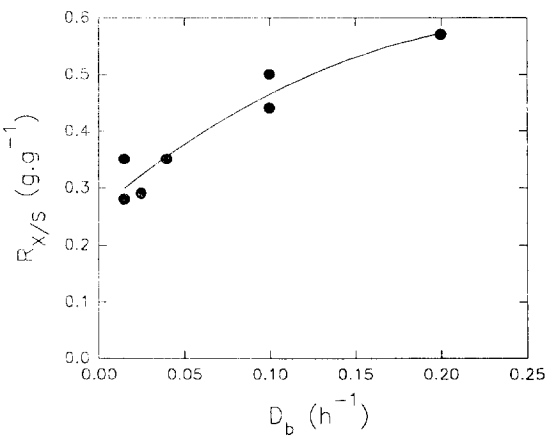

Fig. 3. Influence of the wasting rate on the sludge production yield.

In summary, an increase of the wasting rate in conventional MBR gives an increase both of the biomass viability level and the sludge production yield. In order to minimize sludge production, it is necessary (i) to

amplify maintenance phenomena by imposing low wasting rates or (ii) to accelerate cell death and lysis to perform cryptic growth. This second hypothesis is developed below.

Cell lysis products and consumption in a MBR

Before considering new processes based on cryptic growth to minimize excess sludge volume, a study on cell lysis and its impact on the sludge production yield was investigated. The MBR (see figure 1 with D_5=0) was fed with synthetic wastewater (S_1=1 gCOD.l^{-1}, D_1=0.8 h^{-1}). Cell lysis of biomass extracted from the wasting rate D_4 was performed by mechanical treatment and the raw products or the soluble products were introduced in the MBR at a dilution rate of D_2=0.05 h^{-1}. The wasting rate was D_4=0.1 h^{-1} at which biomass viability was maximal (about 96%). The cultures were grown under synthetic substrate limitation and the cell lysis products were removed in the same time as the synthetic substrate. The results, including biomass growth kinetic,and substrate consumption rates r_S,are presented respectively in figures 4 and 5.

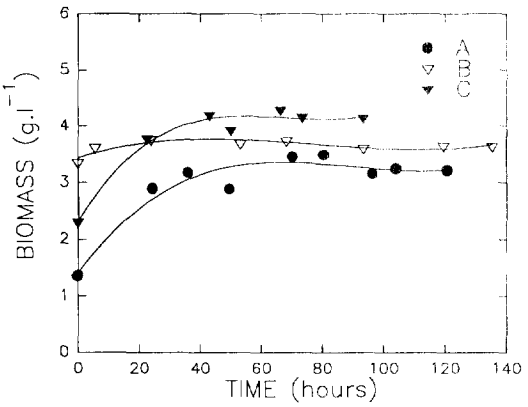

Fig. 4. Growth kinetics of biomass on : (A) synthetic wastewater, (B) synthetic wastewater and the raw cell lysis products (S_2=2.9 $gCOD_{total}$.l^{-1}), and (C) synthetic wastewater and the soluble fraction of the cell lysis products (S_2=2 $gCOD_{soluble}$.l^{-1}).

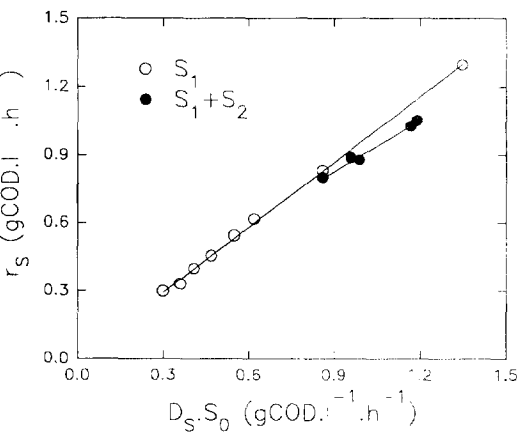

Fig. 5. Consumption rates of (S_1) the synthetic wastewater and (S_1+S_2) a mixture of synthetic wastewater and raw cell lysis products versus the various substrate feed rates.

Concerning the biomass growth (figure 4), the increases of biomass concentration at steady state for the experiments (B) and (C) compared to (A) were respectively 0.3 and 1 g.l^{-1}. The soluble fraction of cell

lysis products was more biodegradable than the particular products. Moreover, in this range of biomass concentration, cell lysis products were not toxic for cryptic growth. Concerning the substrate consumption (figure 5), it appeared that synthetic substrate was completely removed (the slope of the curve represents the purification yield) and raw cell lysis products were partially removed. 50% and 80% respectively of the solid and the soluble fractions of cell lysis products were consumed.

The calculated maintenance coefficients for soluble and total cell lysis products treated mechanically were respectively 0.144 and 0.346 h^{-1}. These coefficients were higher than the one for biomass growing on synthetic substrate and underlined the lower biodegradability of the solid fraction of cell lysis products. The corresponding sludge production yields (table 1) confirmed the benefit of controlling death, lysis and cryptic growth in MBR. The cryptic growth, first introduced by Ryan (1959), has been characterized by Hamer (1985) and Mason and Hamer (1987) for *K. pneumoniae* in batch cultures. These authors proposed a global sludge production yield with cryptic growth of 0.33 $g.g^{-1}$. A similar value was obtained with experiment (B) of table 1. In the case of experiment (C), suspended solids of cell lysis products were removed by external filtration, which allowed the sludge production yield to diminish artificially under the theoretical value.

TABLE 1 EVOLUTION OF THE SLUDGE PRODUCTION YIELD

Experiment	$R_{X/S}$ (gbiomass.g^{-1}COD)
(A) without cell lysis	0.56
(B) with raw cell lysis products	0.36
(C) with soluble fraction of cell lysis products	0.22

These results show that a very low sludge production yield can be obtained. During these experiments, the soluble substrate removal yield was maintained around 95%. Thus a continuous wastewater treatment process with MBR, including sludge digestion after thermal treatment, has been developed.

MBR with thermal treatment : reduction of excess sludge production

Effect of temperature on the cell death rate. Biomass extracted from a continuous culture in conventional MBR (D_1=0.55 h^{-1} and D_4=0.05 h^{-1}) was treated at three different temperatures (50, 70 and 90°C) and the kinetics of cell viability losses were measured by the enumeration method (figure 6).

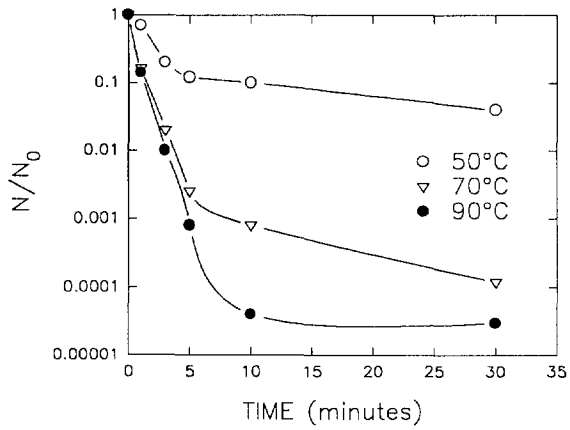

Fig. 6. Death kinetics of *P. fluorescens* at different temperatures.

It appeared that heating the biomass for 10 minutes at 90°C resulted in a biomass viability lower than 0.01%. The reactions of cell death followed first order kinetics with specific death rates of 0.48, 1.06 and 1.21 min^{-1} respectively for treatment at 50, 70 and 90°C. The biomass deactivation energy was evaluated to 26,400 $J.mol^{-1}$ according to Arrhenius' law.

These results indicate that efficient biomass death can be effected at very fast rates by thermal treatment. This treatment induces cell lysis and intracellular product realease phenomena which are characterized below.

Analysis of intracellular products released. Kinetics of intracellular product release were determined using the biomass extracted from MBR cultures (D_4=0.015, 0.03 and 0.05 h^{-1}) and treated at 80, 90 and 100°C for 3 hours. The results expressed as specific release rates of TOC, proteins and polysaccharides are presented in figure 7. Variations of temperature from 80 to 100°C increased the TOC and polysaccharide releases and decreased the protein release (which can be explained by a thermal denaturation). An increase of the net growth rate (or a decrease in sludge age) involved an increase of the TOC release which suggested that with high sludge age, a sludge digestion occurred. These results are in agreement with the experiments of Mason (1986) on microbial lysis and intracellular product release. Moreover, an increase of the net growth rate counter-balanced the ratio of polysaccharide and protein concentrations.

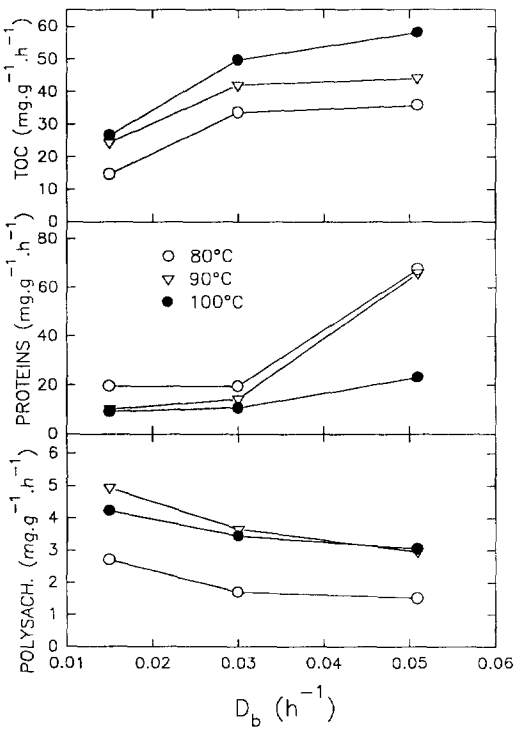

Fig. 7. Specific release rates of intracellular products (TOC, proteins and polysaccharides) for different wasting rates and various temperatures.

These results show that thermal treatment induces biomass death and a partial biomass lysis. The released product concentrations vary in the same way as the net growth rate and are very close for treatments at 90 and 100°C.

MBR associated to sludge thermal treatment. In order to intensify *in situ* biomass death and lysis kinetics to induce biomass growth with the products released, a sludge thermal treatment loop was added to the MBR

(see figure 1 with $D_2=0$ and $D_4=0$). Sludge was heated at 90°C for 3 hours which killed the biomass (close to 100%) and consequently imposed a net growth rate equivalent to D_5 in the MBR. Experiments have been carried out at two different hydraulic dilution rates ($D_1=0.53$ and $0.9\ h^{-1}$) for the synthetic wastewater feed ($S_1=1\ gCOD.l^{-1}$), and respectively at two different net growth rates ($D_5=0.03$ and $0.1\ h^{-1}$). For each experiment, a reference culture was grown for the first 75 hours without thermal treatment ($D_5=0$) and the same wasting rate D_4.

The behaviours of the biomass physiological states are presented in figure 8. With net growth rates of 0.03 and $0.1\ h^{-1}$, the active biomass concentrations were quickly stabilized to 3.9 and $3.6\ g.l^{-1}$ respectively. The regular increase of the total biomass concentrations resulted from the accumulation of dead cells in the first part of the experiments, and biomass residues in the second part. It has been observed that, with thermal treatment, a lysis of dead biomass occurred in the loop at 35% for $D_5=0.03\ h^{-1}$ and 55% for $D_5=0.1\ h^{-1}$; and these percentages of lysis reached 75 and 90% respectively in the MBR for the two net growth rates tested. As previously shown, intracellular products release increased when the net growth rate increased. For example, 1 g of biomass released 1.1 g of COD when grown with a net growth rate of $0.1\ h^{-1}$.

The total biomass removal rates by cryptic growth (table 2) were calculated as the difference between the total biomass production rates (defined as $D_5.X_{total\ initial}$) and the total biomass accumulation rates (extrapolated from total biomass concentration kinetics in figure 8). The same ratio values obtained between the two total biomass removal rates and the two corresponding net growth rates indicated that, in a MBR coupled with a sludge thermal treatment, the biomass death, lysis and cryptic growth kinetics were controlled by the net growth rate.

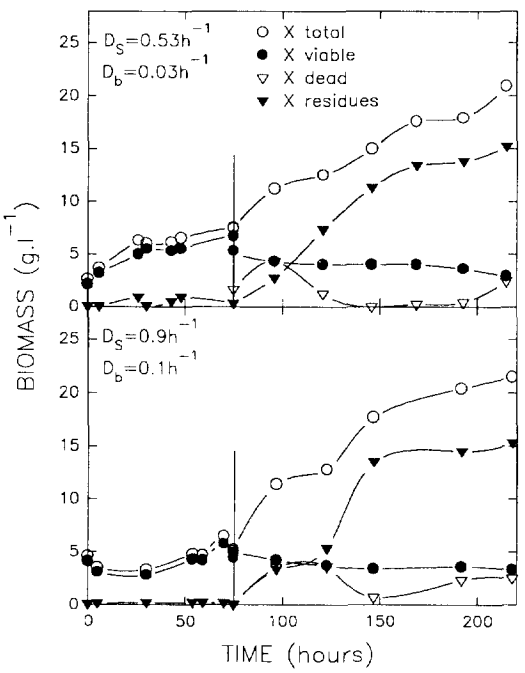

Fig. 8. Evolution of the physiological states of biomass in a MBR, coupled to a sludge thermal treatment after 75 hours working, for two wasting rates and two hydraulic dilution rates.

The results on sludge production for the reference (ref.) and for the sludge thermal treatment (th.t.) cultures are summarized in table 3. The sludge production yield was lower with thermal treatment ($0.17\ g.g^{-1}$ for a net growth rate of $0.1\ h^{-1}$) and decreased when the net growth rate increased. The corresponding maintenance coefficients and limit sludge production yields were $0.104\ h^{-1}$ and $0.19\ g.g^{-1}$ with thermal

treatment compared to 0.035 h^{-1} and 0.57 $g.g^{-1}$ respectively without thermal treatment. The soluble COD removal yield was close to 100% for the two hydraulic dilution rates (D_1=0.53 and 0.9 h^{-1}) and the active biomass concentration was almost the same, which suggested that when the net growth rate increased, the specific biomass activity increased.

TABLE 2 BIOMASS PRODUCTION, ACCUMULATION AND REMOVAL RATES

Net growth rate (h^{-1})	0.03	0.1
r_X production ($g.l^{-1}.h^{-1}$)	0.22	0.52
r_X accumulation ($g.l^{-1}.h^{-1}$)	0.11	0.15
r_X removal ($g.l^{-1}.h^{-1}$)	0.11	0.37

TABLE 3 SLUDGE PRODUCTION RESULTS

Net growth rate (h^{-1})	0.03 (ref.)	0.03 (th.t.)	0.1 (ref.)	0.1 (th.t.)
$R_{X/S}$ ($gX.g^{-1}COD$)	0.35	0.30	0.42	0.17
X_{total} in MBR ($g.l^{-1}$)	6	21	3.6	21.3
X_{active} in MBR ($g.l^{-1}$)	4.2	3.9	3.6	3.6
X_{total} produced after 140 h (g)	25.1	21	50.3	21.3
Soluble COD removal yield (%)	96	94	96	97

These results show that sludge thermal treatment in MBR allows the sludge production yield to decrease significantly by biomass cryptic growth induction, with high biomass metabolic activity and high purification yield. Thus it is possible to integrate partially sludge managment in wastewater treatment by the means of new procedures such as sludge thermal treatment loop in the MBR or the conventional AS processes.

CONCLUSION

Today, with conventional biological wastewater treatments, sludge management (sludge production and disposal) becomes a serious problem. The perfect control of the hydraulic and sludge retention times in a MBR leads one to look at slow biological activities (cell lysis, cryptic growth) and to point out the limitations of the AS process.

It has been shown that when sludge age decreased, the biomass viability and the substrate/biomass conversion yield increased. By obtaining a high active biomass concentration, the MBR allowed one to treat synthetic wastewater loads upto 20 $kgCOD.m^{-3}.d^{-1}$ with a purification yield of about 95%.

An "autodigestive" process was investigated by addition of a thermal treatment of the sludge and the recycling of the hydrolysates to the bioreactor. Thus, improvement of endogenous metabolism was obtained by cryptic growth with both low hydraulic residence time (1 to 2 hours) and low sludge age (10 hours). Because of the sludge thermal treatment, a 3-fold increase of the maintenance coefficient and a 2.5-fold decreaseof the substrate/biomass conversion yield was observed.

Research needs to be conducted on the control of cell lysis and cryptic growth phenomena and to develop new processes, including sludge reduction, with a view to decreasing costs.

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