Excess sludge reduction in activated sludge processes by integrating biomass alkaline heat treatment

M. Rocher*, G. Roux, G. Goma*, A. Pilas Begue***, L. Louvel*** and J.L. Rols***

*Centre de Bioingénierie Gilbert Durand, UMR-CNRS 5504, UR-INRA 792, Institut National des sciences Appliquées, Complexe Scientifique de Rangueil, 31077 Toulouse Cedex 4, France

**LAAS-CNRS, 7 avenue du Colonel Roche, 31077 Toulouse Cedex 4, France

***Rhône Poulenc Industrialisation, Centre de Recherche, d'Ingénierie, de Technologie – Décines, 24 Avenue Jean-Jaurès, 69153 Décines Charpieu Cedex, France

Abstract With new EC regulations, alternative treatment and disposal techniques of the excess sludge produced by activated sludge wastewater treatment plants have to be developed. To decrease activated sludge production yield, microbial cell lysis can be amplified to enhance cryptic growth (biomass growth on lysates). Cell breakage techniques (thermal, alkaline and a combination) were studied to generate *Ralstonia eutropha* (strain model) and waste activated sludge lysates and to evaluate their biodegradability. Gentle treatment conditions by alkaline waste treatment (20 min at 60°C and pH 10 by NaOH addition) allowed waste activated sludge to be solubilized by a two step process (instantaneous and post-treatment) giving a dissolved organic carbon released by the total suspended solids treated of 267 mgDOC.g^{-1}TSS. The biodegradation of the soluble fraction of the lysates by fresh sludge reached 75 and 90% after 48 and 350 hrs of incubation respectively. A validation on a laboratory scale by insertion of a liquor alkaline heat treatment loop in a biological synthetic wastewater treatment process was carried out. A reduction of 37% of the excess sludge was obtained without altering the purification yield of the process.

Keywords Activated sludge; alkaline heat treatment; cryptic growth; sludge reduction; solubilization

Introduction

The activated sludge process is the most widely used biological process for the treatment of domestic and industrial wastewater. This process allows the transformation of dissolved organic pollutants from wastewater into biomass, carbon dioxide and water. Its main by-product is the excess sludge consisting of microbial biomass. Laws concerning wastewater treatment require both economical and safe disposal and result in an increasing production of excess sludge. New stringent EC directives concerning waste activated sludge (WAS) disposal require solutions to reduce or eliminate excess sludge.

Sludge production depends on different factors: biodegradability of the organic pollutant, mass loading of the treatment plant (Eckenfelder, 1978), degradation of microbial cells by endogenous respiration or cellular lysis (Bouillot *et al.*, 1990) and ingestion of bacteria by predators (Lee and Welander, 1996). One concept for decreasing sludge production involves the reduction in the global substrate to biomass conversion yield. It can be obtained by amplifying microbial cell lysis and generating biomass growth on the lysis products which is defined as the cryptic growth. Cell disruption can be provoked by chemical, physical, mechanical or biological methods (Misselberg, 1995).

Some treatments of cellular lysis have already been integrated into the activated sludge process. One consisted in the ozonation of a fraction of recycled sludge before being returned to the aeration tank (Yasui and Shibata, 1994; Yasui *et al.*, 1996): one third of the ozonated sludge was mineralised via the treatment. Another example is the association of a continuous biomass thermal treatment system (100°C, 3 hours) with a membrane bioreactor for synthetic wastewater treatment by *Pseudomonas fluorescens* (Canales *et al.*,

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1994). This process amplified biomass breakage and cryptic growth and resulted in a 2.5 fold decrease in the global biomass to substrate conversion yield.

A method to reduce excess sludge production, based on cryptic growth, seems to be reliable and realistic. To be economically viable, cell breakage must be generated by gentle treatment conditions. The aim of this paper is to define conditions of deactivation and lysis of WAS by various cell disruption techniques, and to evaluate the biodegradability of the released organic matter. A validation on a laboratory scale of a liquor alkaline heat treatment reactor coupled to a biological synthetic wastewater treatment process is presented.

Materials and methods

Microorganisms and culture medium

Ralstonia eutropha ATTC 17697 was used as a strain model to evaluate the behaviour of bacteria toward cell disruption techniques. *R. eutropha* can be found at 4 to 40% in the WAS (Thomazeau, 1982). The culture medium of *R. eutropha* consisted of mineral salts (Johnson and Stanier, 1971) and benzoate (concentration of dissolved organic carbon (DOC) 3.6 $g.l^{-1}$. *R. eutropha* was cultivated in a 3 litre liquid volume bioreactor (Applikon) working as a chemostat (hydraulic dilution rate of 0.09 h^{-1}) at 30°C, pH 7.5 (regulated by H_3PO_4 supply), and dissolved oxygen concentration maintained at 50% of the air saturation via agitation rate variation. This allowed physiologically stable microbial samples to be used.

The WAS used in this study was collected from the aeration tank of the domestic wastewater treatment plant of Toulouse-Ginestous. Sludge was collected the day of the experiment and was aerated for 4 hours before being used in order to obtain a residual DOC lower than 20 $mg.l^{-1}$. The total suspended solids (TSS) concentration is 3 $gTSS.l^{-1}$, the volatile suspended solids (VSS) represent 83% of TSS and the mass loading of the plant is 0.45 $kgBOD_5.kg^{-1}$ $TSS.d^{-1}$, where BOD_5 is the biological oxygen demand. The culture medium of WAS consisted of mineral salts (Rocher *et al.*, 1999) and acetate (concentration of 3.9 $gDOC.l^{-1}$). WAS was cultivated in a 3 litre liquid volume bioreactor (Applikon) working as a chemostat (hydraulic dilution rate of 0.02 h^{-1}) at 30°C, pH 7.5 (regulated by H_3PO_4 supply), and dissolved oxygen concentration maintained at 50% of the air saturation via agitation rate variation. As presented in Figure 1, the bioreactor was coupled with an alkaline heat treatment reactor by using a liquid recycling loop. The liquor treatment conditions were a temperature of 60°C, a pH of 10 (addition of NaOH 1 M) and a residence time of 20 min. The operating conditions were related to the liquor recycling rate varying from 0 to 15.

Experimental conditions

To induce cell breakage and organic matter solubilization, *R. eutropha* or WAS cells were treated by means of various disruption techniques:

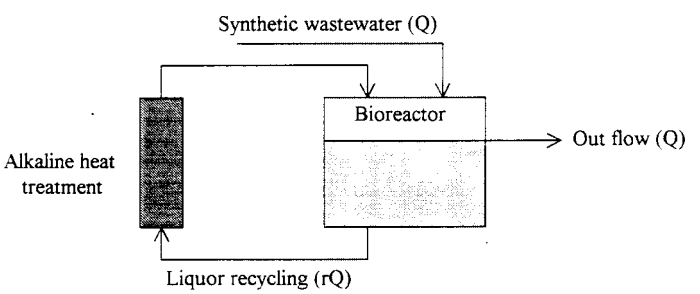

Figure 1 Configuration of the laboratory pilot plant. The bioreactor was operating as a chemostat with a hydraulic dilution rate of 0.02 h^{-1}. The alkaline heat treatment reactor was coupled to the bioreactor.

- thermolysis (20 to 100°C), with glass tubes (1 to 100 ml) in water-bath and duration from 2 to 60 min,
- chemical hydrolysis by NaOH addition. Variables considered in the hydrolysis experiments were pH (from 7.4 to 12) and temperature (from 30 to 100°C) in water-bath.

The biodegradability tests of the lysate soluble fraction (centrifugation at 42 000 g for 10 min) produced as above were carried out in two litre conical flasks continuously stirred at a temperature of 30°C and the pH was initially adjusted to 7.5 by phosphate buffer addition. The flasks contained 100 ml of the lysate soluble fraction and 100 ml of the mineral salts (without organic carbon source), and were inoculated at 10% v/v by the supernatant of fresh WAS (allowed to settle for 30 min). The incubation period was 350 hours.

Analytical procedures

TSS concentration was expressed as dry weight and determined as follows: known volumes of culture medium were withdrawn at various intervals and filtered through a 0.2 µm-pore-size polyamide membrane to recover TSS. The membranes were then dried at 60°C under vacuum for 24 hours.

Oxygen consumption rates were measured by a respirometric method using Clark electrodes (YSI, model 5 300). The suspension of unwashed *R. eutropha* cells (100 µl at 2.4 gTSS.l^{-1}) was transferred from the chemostat into the respirometric cell maintained at a constant temperature and containing 7 ml of culture medium (benzoate and mineral salts) which was initially aerated to increase the dissolved oxygen concentration to 6–8 mg.l^{-1}. After the aeration was stopped, a linear decrease in oxygen concentration allowed the total respiration rate under non-limiting substrate consumption conditions to be calculated. Biomass viability was performed by enumeration on Petri dishes by the drop-count method with nutritive medium (Biokar Diagnostics). The incubation period was 872 hours at 30°C (Fung and Kraft, 1968). DOC of the soluble fraction of the culture samples (centrifugation 10 min at 42 000 g) was determined by a Dohrmann DC-180 carbon analyser (Roosemount Analytical, California).

Results and discussion

This section is divided into three parts: first *R. eutropha* cell physico-chemical treatment, followed by the WAS physico-chemical and biodegradation of the so-generated lysates and finally the coupling of alkaline heat treatment reactor to a biological wastewater treatment process.

R. eutropha physico-chemical treatments

The effect of temperature on *R. eutropha* cells was evaluated by respirometric measurements. The respiration rates (r_{o2}) were measured and the specific respiration rates ($q_{o2} = r_{o2}$/TSS) calculated are presented in Figure 2. On the basis of the specific respiration rates, the optimum growth temperature was 30°C. For temperatures lower than 30°C, the activation energy was +11.9 kcal.mol^{-1}. For temperatures higher than 30°C, the deactivation energy was –59.1 kcal.mol^{-1}. A complete cell deactivation was obtained for temperatures higher than 55°C. Two minutes were sufficient to inactivate cells at a temperature of 60°C. This temperature threshold on cell deactivation was confirmed by another biomass viability determination technique: cultivation of treated cells (2 min at 60°C) in liquid culture medium for 15 days.

Cell lysis was evaluated by the release of DOC from the treated biomass. Fresh *R. eutropha* cells were incubated at various temperatures (60 and 100°C) and incubation periods (2 to 180 min), and then the released DOC was measured. The results are presented in Figure 3A. For each temperature tested, the kinetic behavior of the release of DOC was

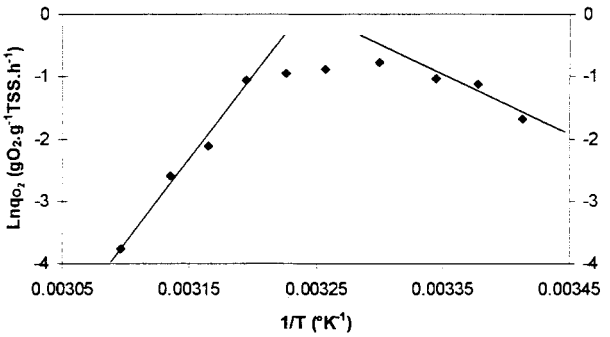

Figure 2 Evaluation of temperature effects on *R. eutropha* cells through the specific respiration rate q_{o2} *R. eutropha* cells were cultivated on benzoate (2.4 gTSS.l^{-1}) at 30°C and transferred into the respirometric cells at various temperatures (between 20 and 55°C) for an incubation period of 15 min.

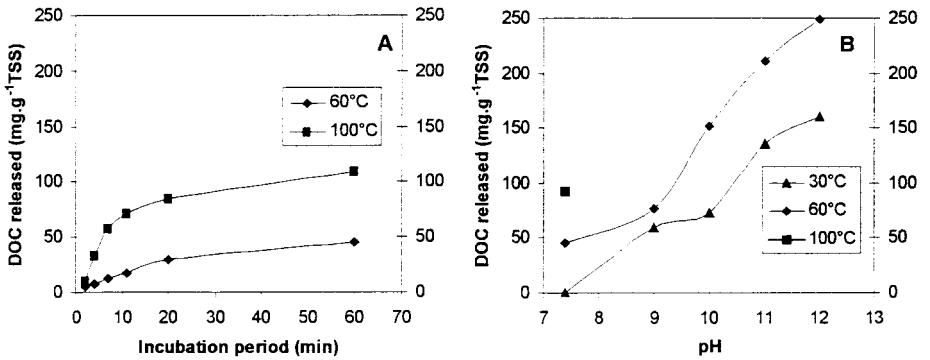

Figure 3 DOC released in the liquid medium by treated *R. eutropha* cells. *R. eutropha* cells were cultivated on benzoate (2.4 gTSS.l^{-1}) at 30°C and transferred into a water-bath at various temperatures. A: temperature effects with an incubation period varying from 2 to 60 min. B: combined effects of temperature and pH (NaOH addition) with an incubation period of 30 min

the same: after 20 min of incubation, 80% of the maximum releasable DOC was obtained. Beyond one hour of treatment (up to 3 hours), no additional release was observed (data not shown). For any given incubation period, the released DOC increased with temperature. One hour of incubation gave 47 and 110 mgDOC.g^{-1} TSS of release at 60 and 100°C respectively.

In order to increase the level of cell solubilization, combinations of temperature and alkaline agent were tested. *R. eutropha* cells were incubated for 30 min at various temperatures and pH values (NaOH addition, pH from 7.4 to 12). The temperatures chosen were 30°C (optimal temperature for biomass growth), 60°C (threshold for complete cell deactivation) and 100°C (maximum temperature investigated for our study). The results are presented in Figure 3B. Temperature and pH rises resulted in an increase in the released DOC. For a temperature of 30°C, the released DOC was due to the actual effect of pH. At a temperature of 30°C and a pH above 10.5, the released DOC was higher than that obtained by a thermal treatment alone (100°C). The value of combining temperature and chemical treatment for cell lysis was illustrated by the curve obtained at 60°C and various pH. For values within the range of temperature and pH explored, the maximum release of DOC was 250 mgDOC.g^{-1} TSS obtained at 60°C and pH 12. With intermediary conditions of pH 10 and a temperature 60°C, the released DOC (150 mgDOC.g^{-1}TSS) was higher or equivalent respectively to that obtained by thermal treatment at 100°C and neutral pH, or by alkaline treatment at pH 12 and 30°C. In the light of these results, alkaline heat treatment was the most efficient process to induce cell lysis under gentle operating conditions.

WAS physico-chemical treatment and biodegradation

In the light of the results obtained with a pure microbial strain, the cell disruption technique was applied to WAS mixed microbial population and cryptic growth was evaluated on the basis of DOC degradation. WAS collected in a municipal wastewater treatment plant was treated at 60°C, pH 10 for 20 min and the release of DOC was quantified instantaneously after the treatment (instantaneous cellular lysis), or after a 15 day sterile incubation of the insoluble fraction (centrifugation at 42 000 g for 10 min) of the treated WAS (post-treatment cellular lysis). The results are presented in Table 1. The instantaneous release of DOC 150 mgDOC.g^{-1}TSS and the post-treatment release of DOC was 117 mgDOC.g^{-1}TSS. Thus, the total released DOC (instantaneous and post-treatment) by WAS with the investigated treatment was 267 mgDOC.g^{-1}TSS. The released DOC reaction was a distinct two phase process: first, a rapid step, then a slower second phase. Similar results were reported by Rajan *et al.* (1989) for alkaline hydrolysis of WAS at low temperature (lower than 38°C).

A comparison of our results with other experiments on sludge disintegration before anaerobic digestion can be made. The use of ultrasound (3.6 kW, 31 kHz, 64 sec) allowed a chemical oxygen demand (COD) solubilization to be obtained from raw sludge of 230 mgCOD.g^{-1}TSS (Tiehm *et al.*, 1997). A combination of simultaneous alkaline (40 meq.l^{-1} of NaOH) and ultrasound (120 W, 20 kHz, 14.4 sec.ml^{-1}) pre-treatment enabled 78% of total COD from WAS to be solubilized (Chiu *et al.*, 1997). Finally, a mechanical cell disintegration technique using a stirred ball mill or a high pressure homogenizer with a high specific energy consumption (10 000 kJ.kg^{-1}) allowed 90% of the total COD from WAS to be solubilized (Kopp *et al.*, 1997). All of these results can not be directly compared with our results, the parameters used to quantify the disintegration processes being different.

In processes involving a reduction in excess sludge production, the lysate biodegradability (cryptic growth) is the most important parameter. This concept has been developed in the literature from work on thermophilic digestion (Mason, 1986; Mason *et al.*, 1986a and b; Aoki and Kawase, 1991). *P. fluorescens* cell auto-digestion (Canales *et al.*, 1994), WAS cell auto-digestion after ozonation (Yashui and Shibata, 1994; Yashui *et al.*, 1996) and aerobic storage (Gaudy *et al.*, 1967; Urbain *et al.*, 1993). As cryptic growth consists in the ability of microorganisms to assimilate cell intracellular products, verification of the lysates biodegradability was carried out, first with the soluble fraction of the instantaneously released DOC and then with the soluble fraction of the post-treatment released DOC. These fractions were separately inoculated with fresh WAS. The results of DOC degradation after 48 and 350 hours of incubation are presented in Table 1. The DOC degradation yields were higher than 85% after 350 hours of incubation. A large part of the degraded DOC, higher than 70%, was removed after an incubation period of 48 hours. It should be noted that up to 60% of the biomass was solubilized by alkaline heat treatment for a biomass chemical composition of 45.7% of carbon. This can result, for a DOC biodegradation level of 90%, in a decrease in the global yield of sludge production by 33% (based on carbon to biomass conversion yield of 1.1 mgTSS.g^{-1}DOC, Egli and Fiescher, 1981).

Table 1 Alkaline heat treatment (60°C and pH 10 for 20 min) of WAS (2.6 gTSS.l^{-1}). Biomass solubilization was evaluated by the measurement of DOC released (instantaneous and post-treatment cellular lysis). Biodegradability of the instantaneous and post-treatment released DOC was evaluated after 48 and 350 hrs of incubation with supernatant of settled fresh activated sludge in conical flasks.

	DOC release (mgDOC.g^{-1}TSS)	DOC degradation yield (%)	
		After 48 hrs	After 350 hrs
Instantaneous release	150	76	89
Post-treatment release	117	72	87

Integrated biological synthetic wastewater treatment

A laboratory scale pilot version of the proposed sludge lysis system has been constructed: it consists of a biological wastewater treatment bioreactor operating as a chemostatic (hydraulic dilution rate of 0.02 h^{-1}), inoculated with fresh WAS, fed with a synthetic effluent containing acetate as the only organic carbon source (concentration of 3.9 $gDOC.l^{-1}$, which represents a high volumic loading of 1.87 $kgDOC.m^{-3}.d^{-1}$) and coupled with an alkaline heat treatment reactor (60°C, pH 10, 20 min). The operating conditions related to the liquor recycling rate and the volume of the treatment reactor are given in Table 2. For each liquor recycling rate, steady state was obtained after the bioreactor volume had been renewed six times by fresh synthetic effluent. The experiments were carried out in the sense of increasing liquor recycling rate with three references ($r = 0$) at the beginning, the middle and the end.

The results of these experiments are presented in Figure 4. With the increase in the liquor recycling rate from 0 to 4, a decrease in the total and viable biomass concentrations was observed (Figure 4A). The decrease in the viable biomass concentration was due to the deactivation process by the alkaline heat treatment, whereas the decrease in the total biomass concentration resulted from the biomass solubilization by the alkaline heat treatment and the cryptic growth occurring in the bioreactor. Beyond this threshold ($r = 4$), the concentrations remained stable. For these bioreactor operating conditions, a recycling rate of 4 (for which the biomass is exposed 4 times to the alkaline heat treatment during its stay in the bioreactor) enabled a lower excess sludge production to be obtained, with a reduction of 37% compared to the reference culture (Figure 4B). This limit in the reduction level is certainly imposed by the biomass solubilization capacity of the sludge treatment used. In spite of the processes of biomass solubilization and cryptic growth which occurred consecutively, the purification yield (DOC removal) of the bioreactor remained stable, around 93% ± 3. So, with increasing liquor recycling rate, the specific DOC removal rate was increasing because of the decrease in the biomass concentration (viable and total) and the increase of the DOC loading (recycled soluble fraction of the biomass lysates generated).

Table 2 Operating conditions of the laboratory pilot plant through the liquor recycling rate (r) and the corresponding volume ratio between the alkaline heat treatment reactor and the bioreactor (V_T/V_B)

r	0	1.65	2.5	4	7.5	15
V_T/V_B (%)	0	1.1	1.7	2.7	5	10

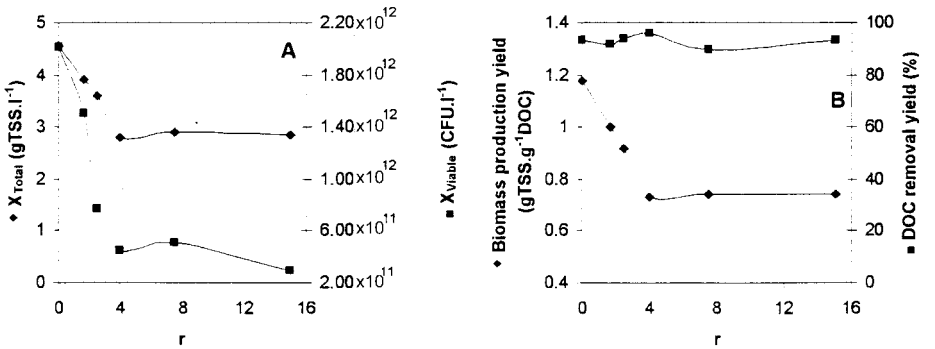

Figure 4 Steady state results of the laboratory pilot plant operating at various liquor recycling rates (r between 0 and 15). The bioreactor was fed with a synthetic wastewater (3.9 $gDOC.l^{-1}$ of acetate, flow rate $Q = 0.061\ l.h^{-1}$) and the alkaline heat treatment reactor was maintained at 60°C and pH 10 (NaOH addition) with a liquor residence time of 20 min

These results show that sludge alkaline heat treatment allows the sludge production yield to be decreased significantly by biomass cryptic growth induction, with high biomass metabolic activity and high purification yield. Thus it is possible to integrate partially sludge management in wastewater treatment by means of new procedures such as sludge alkaline heat treatment loop in the conventional activated sludge processes.

Conclusion

Conventional activated sludge processes for wastewater treatment transform the organic pollution into gas, water and biomass. The cost of the excess sludge treatment and disposal can represent up to 60% of the total operating costs. A recirculation process which had the ability to reduce WAS should result in cost benefits by diminution or elimination of the classical sludge post-treatments (dewatering, incineration methods, landfilling, etc.). A combined thermal alkaline WAS treatment can provide a viable alternative to other sludge treatment processes. The optimal conditions to induce cell breakage and to produce biodegradable lysates were found to be a pH of 10 (NaOH addition) at a temperature of 60°C for 20 min. At a relatively low alkaline level, a sufficient drop in pH was achieved, and eliminated or reduced the need for expensive chemical neutralization. The coupling of this WAS treatment system to a biological wastewater treatment bioreactor allowed a 37% reduction in the excess sludge production to be obtained. To extrapolate the results of this study to a process application, the energetic aspects and the necessary alkaline agent quantity should be taken into account.

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