

## Aerobic Biodegradation of Precoagulated Cheese Whey Wastewater

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**ABSTRACT:** Prior to the application of an aerobic biological process, cheese whey wastewater has been pretreated by means of a precipitation stage by adding either NaOH or CaOH<sub>2</sub>. Both precipitating agents reduce roughly 50% of the raw wastewater chemical oxygen demand (COD). The sludge generated in the prestage shows acceptable settling properties, although solids from the CaOH<sub>2</sub>-treated effluent are better separated from the liquid bulk than those formed in NaOH-processed wastewater. In both situations, the presedimentation stage renders a supernatant more prone to biodegradation than the untreated effluent. The previous statement is corroborated by the determination of some biological kinetic parameters. Under the operating conditions used in this work, sludge generation after the biological process is reduced to a minimum. The sludge generated shows good settling properties, especially for those experiments in which CaOH<sub>2</sub> has previously been added.

**KEYWORDS:** cheese whey wastewater, pretreatment, dairy effluents, sedimentation, aerobic biodegradation

### INTRODUCTION

Within the food industry, the dairy industry is one of the major contributors to wastewater generation in terms of both organic load value and effluent volume.<sup>1–3</sup> Dairy wastewater consists mainly of raw materials lost during handling and processing and cleaning materials carried into the processing water. The highly variable nature of dairy wastewaters in terms of volume, pH, and organic and suspended solids contents makes the choice of an effective wastewater treatment regimen difficult. Without proper treatment, this wastewater can pose potential environmental hazards. As regulations become more stringent and surcharges to dispose of dairy wastewater become more expensive, selected treatment should meet the required demands and reduce both costs. Broadly speaking, dairy wastewater composition involves a substantial concentration of fat, milk, protein (mainly casein), lactose, lactic acid, minerals, detergents, and sanitizers.<sup>4</sup>

With increasing social awareness about the environment, the dairy industry is forced to treat its effluents effectively and efficiently before disposal into the public drainage. The challenge is to find cost-efficient and environmentally sustainable approaches to water reuse, byproduct disposal, and waste management.

Biological processes are normally recommended to deal with this type of effluent.<sup>5–7</sup> Thus, dairy ponds use anaerobic treatment as a means of treating the wastewater. Anaerobic conditions are obvious from the continuous amount of gas bubbles off-gassing from the pond surface. However, this process tends to cause odors, which can be a source of complaints. Additionally, odor-generating ponds are prime spots for mosquitoes to live and grow, causing a further nuisance. The conversion of the dairy pond from an anaerobic system to an aerobic process creates a completely different environment. The use of surface aerators forces air below the water surface to add oxygen and mix the wastewater and settled sludge. The added oxygen creates a bacteria-friendly environment; therefore, the time involved in solids degradation is greatly reduced. Aerobic systems can be an alternative to small–medium factories where energy demand is

not an important drawback commonly associated with aerobic biodegradation processes.

Despite the significant biodegradability of dairy effluents, high detention times are normally required in aerobic processes.<sup>8</sup>

Physicochemical pretreatment is envisaged as an important stage for wastewater containing particles or debris that could damage downstream pumps or equipment. Pretreatment can also reduce the concentration of nonbiodegradable and/or biorecalcitrant chemical oxygen demand (COD), rendering, therefore, an effluent more susceptible to biotreatment.<sup>9,10</sup>

A survey of some scientific databases reveals that most works dealing with cheese wastewater are focused on biodegradation stages with no integration with supplementary steps. Improvement of the biological process is an attractive option in small and medium cheese-manufacturing industries where biological ponds are size limited. Use of more effective and economic coagulant reagents (as is the case of Ca(OH)<sub>2</sub>) as an alternative to the conventional ferric or aluminum salts is one of the targets of this study. Thus, this work is aimed at establishing the influence of an alkaline pretreatment on the aerobic biodegradation of a whey wastewater from the production of Serpa cheese. In this sense, a simplistic modeling of the biodegradation process is used for comparison purposes.

### MATERIALS AND METHODS

**Cheese Whey Wastewater (CWW).** CWW was collected from a small industry located in the Alentejo region of Portugal. This small plant produces the so-called “Serpa cheese” typical of this area in southern Portugal. Wastewater was characterized and immediately frozen to avoid biological activity. The main properties of this effluent

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**Table 1. Average Parameter Removal (Duplicated Experiments) after Treatment of Raw Whey Wastewater with FeCl<sub>3</sub> (FeCl<sub>3</sub>-PTted), NaOH (NaOH-PTted), or CaOH<sub>2</sub> (CaOH<sub>2</sub>-PTted)**

parameter	raw WW	NaOH-PTted (% removal)	CaOH <sub>2</sub> -PTted (% removal)	FeCl <sub>3</sub> -PTted <sup>a</sup> (% removal)
COD (mg L <sup>-1</sup> )	18500 ± 1400	50.0	46.6	32.1
BOD (mg L <sup>-1</sup> )	14800 ± 1500	46.0	54.8	23.4
turbidity (NTU)	1331 ± 219	79.6	76.0	72.1
total solids (mg L <sup>-1</sup> )	7650 ± 600	-43.0	-46.0	21.7
absorbance 220 nm <sup>b</sup>	0.878 ± 0.105	70.0	48.9	35.2
absorbance 254 nm <sup>b</sup>	0.334 ± 0.045	70.0	90.1	41.6
chloride (mg L <sup>-1</sup> )	2407 ± 248	7.9	0.5	-12.5
nitrogen (mg L <sup>-1</sup> )	337.7 ± 18.4	34.4	55.0	19.6
N-NH <sub>3</sub> (mg L <sup>-1</sup> )	56.4 ± 7.3	33.9	0.0	0.0
phosphorus (mg L <sup>-1</sup> )	6.6 ± 0.3	45.9	96.5	0.0
proteins (μg L <sup>-1</sup> )	943 ± 6	00.0	93.5	6.4
lactose (mg L <sup>-1</sup> )	180 ± 3	34.4	76.1	0.0

<sup>a</sup> From ref 4. <sup>b</sup> After 1:50 dilution with ultrapure water.

are displayed in Table 1. A detailed description of the effluent can be found elsewhere.<sup>8</sup>

**Analytical Procedures.** COD was monitored by the absorbance of the sample after dichromate digestion at 150 °C for 2 h in the presence of silver and mercury sulfates. Biological oxygen demand (BOD) was obtained by a respirometric method (WTW OxiTop) under controlled conditions of temperature, agitation, and light absence. Microorganisms were taken from a municipal wastewater treatment plant and acclimated to the effluent. Turbidity was measured by a WTW Turb550 turbidimeter. Solids were analyzed after filtration of a determined volume of sample. Total suspended solids (TSS) were obtained from the residue in the filtration paper (Whatman 934-AH), whereas total dissolved solids were calculated after evaporation of the filtrate. Absorbance at different wavelengths was measured in an Ultrospec 2100pro spectrophotometer. Both pH and redox potential were monitored in a WTW InoLab apparatus (pH electrode SenTix 41 and redox electrode SenTix RP). Conductivity was obtained in a JENWAY 4510 conductivity meter. Total phosphorus was quantified by the absorbance of the sample after reaction of orthophosphates and a solution of vanadate–molybdate. Oil and fats were determined after extraction by means of a Soxhlet apparatus using petroleum ether as the extracting agent. Chlorides were titrated with silver nitrate in the presence of potassium chromate. Lactose was analyzed by means of the Tell reagent. Total proteins were monitored by using Lowry's method. This method is based on the blue coloration of a complex formed in the reaction of Folin's reagent and proteins in alkaline conditions and in the presence of copper as catalyst. Ammonium and Kjeldahl nitrogen were quantified by following the standard methods. References of the different analytical procedures can be found elsewhere.<sup>8</sup>

**Reagents and Procedure.** Sedimentation experiments were carried out in a Jar Test apparatus (Olabo ISCO) equipped with six-speed regulated stirrers. Runs were completed in 0.4 L beakers under initial fast agitation (150 rpm) for 2 min and further slow agitation (20 rpm) for 20 min.

Sedimentability tests were conducted in a normalized 1 L glass probet 34 cm high. Some runs were also conducted in a 13 L column (150 cm high, 10 cm diameter) equipped with several sampling ports located at different positions on the column.

Aerobic biodegradation experiments were conducted in a 2 L glass reactor continuously oxygenated by an air flow stream. Microorganisms were taken from a municipal wastewater plant and acclimated to the effluent for 5 days.

Chemicals used in this study were acquired from Aldrich and used as received.

## RESULTS AND DISCUSSION

**Alkaline Pretreatment. NaOH Addition.** NaOH was added to raw whey wastewater to achieve a precipitation pH value in the range of 11–12.5. In this narrow pH range no significant differences were observed. Accordingly, for economic reasons (lower NaOH requirements), replicas of experiments were conducted at the lowest tested pH of 11. Table 1 shows the removal efficiencies obtained for different parameters in comparison to values determined in the raw whey wastewater.

As inferred from this table, with some exceptions, removal efficiencies are higher than those reported in a previous work<sup>8</sup> by using FeCl<sub>3</sub> as precipitating agent (FeCl<sub>3</sub>-PTted WW). FeCl<sub>3</sub> pretreatment has also been shown to adequately render the raw whey wastewater more biodegradable. Another point to highlight is that both COD and BOD are similarly removed after the precipitation stage, so the biodegradability index, measured as the ratio BOD/COD, remains sufficiently high to apply a post biological stage.

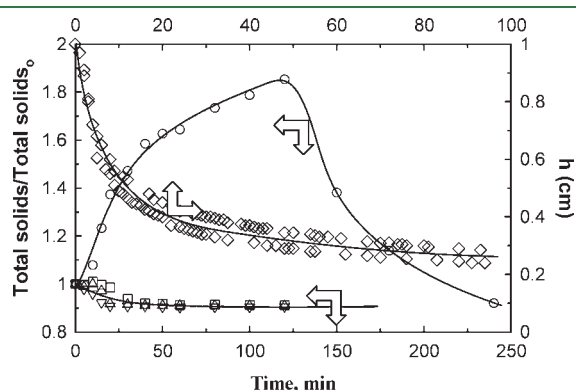
With regard to the solid separation step, when NaOH was used as precipitating agent, the sedimentation was flocculent; that is, no clear distinction is observed between the cleared supernatant and the solids. The profiles of solid concentration versus time for different heights in a cylindrical column (30 cm diameter × 150 cm height) are shown in Figure 1. Solids at the top of the column are continuously removed throughout the whole process. In contrast, solids start to accumulate in those zones located at the column bottom until a maximum in concentration is achieved; thereafter, the sedimentation velocity is faster than the accumulation rate. In any case, it seems that solid sedimentation properties must be enhanced. Thus, the addition of flocculants can be suggested.

**Ca(OH)<sub>2</sub> Addition.** CaOH<sub>2</sub> was added in the pH interval from 10.5 to 13.0, and again no significant differences were found for runs conducted at different initial pH values. Once more, Table 1 shows an acceptable performance of the coagulation process with values comparable to the other two precipitating agents shown in the table. However, settling properties of the sludge formed are significantly better than those experienced when FeCl<sub>3</sub> or NaOH was added to raw wastewater. Zone settling type sedimentation was obtained for CaOH<sub>2</sub>-PTted effluents (Figure 1).

A rough estimation of the reagent needs associated with the previous processes indicates that 2.7 g of NaOH is needed to shift

the pH of 1 L of raw wastewater to 11.0. Additionally,  $\text{CaOH}_2$  requirements are located in the range of  $3.8\text{--}5.0\text{ g L}^{-1}$  depending on initial pH. By taking into consideration that  $\text{FeCl}_3$  price doubles the lime price,<sup>11</sup> the use of  $\text{CaOH}_2$  is recommended as a pretreatment stage.

**Aerobic Biodegradation.** *Kinetics of the Aerobic Biodegradation.* Aerobic biodegradation experiments of raw and pretreated effluents were carried out at different initial substrate/biomass ratios. For comparison purposes, some additional biodegradation experiments were also carried out by using  $\text{FeCl}_3$ -pretreated wastewater ( $\text{FeCl}_3$ -PTted).



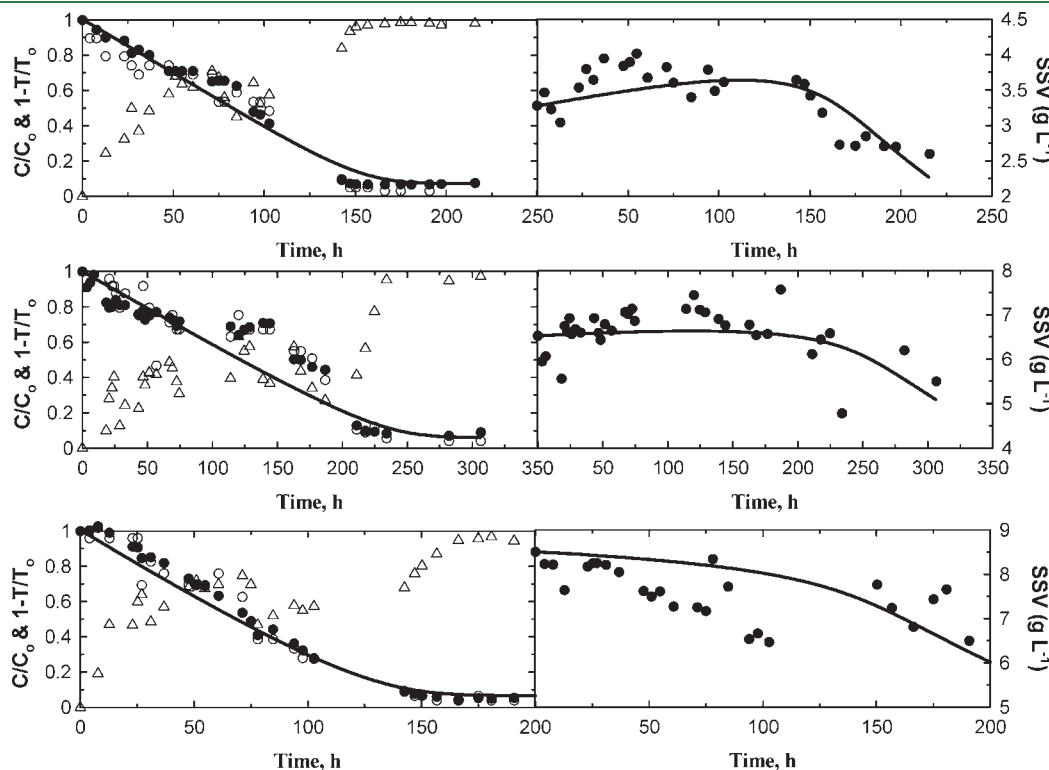
**Figure 1.** Settling characteristics of solids precipitated after  $\text{NaOH}$  or  $\text{CaOH}_2$  addition to raw whey wastewater: normalized solids concentration in a column at different heights ( $\text{NaOH}$  addition) ( $\circ$ ,  $-140\text{ cm}$ ;  $\square$ ,  $-120\text{ cm}$ ;  $\triangle$ ,  $-40\text{ cm}$ ;  $\nabla$ ,  $-20\text{ cm}$ ). Solid-liquid interface height ( $h$ ) when  $\text{CaOH}_2$  is added is represented by  $\diamond$ .

Figure 2 shows the results obtained when raw wastewater was treated in an aerobic batch process. As observed from this figure, COD and BOD follow similar trends, indicating the biodegradable nature of the raw wastewater. Both parameters (COD and BOD) seem to linearly decrease until a roughly 90–95% removal is obtained. Thereafter, the biodegradation process comes to a halt. Accumulation of nonbiodegradable material is likely to occur at the end of the reaction period. Another point to highlight is the rapid and efficient elimination of turbidity with reductions close to 98–99% when the process is finished.

Depending on the operating conditions, COD accumulation is experienced after approximately 150–200 min from the start of the biodegradation. In no case was total COD elimination attained. Consequently, the concept of residual COD was used in assessing the biological treatability of this effluent.<sup>12</sup> According to the model proposed by Orhon and co-workers,<sup>12</sup> initially, substrate (measured as COD) can be catalogued as readily biodegradable ( $\text{COD}_B$ ) and biorecalcitrant or inert ( $\text{COD}_I$ ) to the microbial community. The latter is not removed in the process and bypasses the treatment system. Additionally, part of the remaining COD at the end of the experiments can be generated in the course of the microbial growth and decay stages ( $\text{COD}_M$ ). Accordingly, the final effluent can be constituted by three COD fractions:

$$\text{COD}_{\text{total}} = \text{COD}_B + \text{COD}_I + \text{COD}_M \quad (1)$$

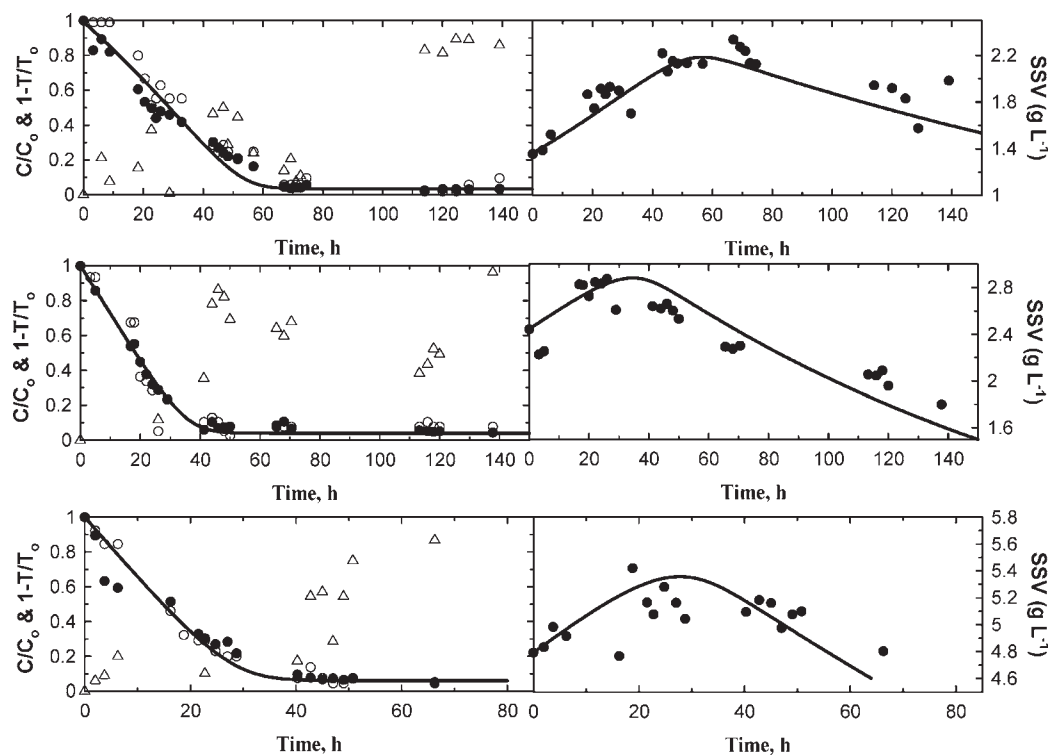
The different COD fractions are not distinguished in the COD analytical procedure. Usually,  $\text{COD}_B$  is completely removed in the biodegradation process, whereas the remaining COD



**Figure 2.** Aerobic biodegradation of raw whey wastewater. Experimental conditions at  $T = 290\text{ K}$  and  $\text{pH } 7.5$ , COD ( $\text{mg L}^{-1}$ ), BOD ( $\text{mg L}^{-1}$ ), turbidity (NTU), and  $X_{A0}$  ( $\text{mg L}^{-1}$ ): (top) 11358, 9700, 658, and 3280; (middle) 13150, 12700, 575, and 6528; (bottom) 8983, 7500, 342, and 8510, respectively. Symbols:  $\bullet$ , COD ( $X_A$  in right-hand figures);  $\circ$ , BOD;  $\triangle$ , turbidity ( $T$ ) removal ( $1 - T/T_0$ ). Lines = model calculations.

Table 2. Kinetic Parameters for the Aerobic Biotreatment of Cheese Whey Wastewater

wastewater	$\mu$	$Y_H$	$K_S$	$Y_M$	$b$
raw	$0.004 \pm 0.001$	$0.440 \pm 0.01$	$800 \pm 40$	$0.065 \pm 0.005$	$0.005 \pm 0.002$
FeCl <sub>3</sub> -PTted	$0.015 \pm 0.002$	$0.25 \pm 0.05$	$800 \pm 50$	$0.040 \pm 0.005$	$0.005 \pm 0.001$
CaOH <sub>2</sub> -PTted	$0.019 \pm 0.003$	$0.35 \pm 0.05$	$820 \pm 80$	$0.040 \pm 0.005$	$0.005 \pm 0.001$
NaOH-PTted	$0.02 \pm 0.002$	$0.25 \pm 0.05$	$800 \pm 60$	$0.040 \pm 0.005$	$0.008 \pm 0.001$



**Figure 3.** Aerobic biodegradation of FeCl<sub>3</sub>-PTted wastewater. Experimental conditions at  $T = 290$  K and pH 7.5, COD ( $\text{mg L}^{-1}$ ), BOD ( $\text{mg L}^{-1}$ ), turbidity (NTU), and  $X_{A0}$  ( $\text{mg L}^{-1}$ ): (top) 6420, 4700, 31.2, and 1360; (middle) 4840, 3640, 91.8, and 2450; (bottom) 4775, 3700, 52.9, and 4790, respectively. Symbols: ●, COD ( $X_A$  in right-hand figures); ○, BOD; △, turbidity ( $T$ ) removal ( $1 - T/T_0$ ). Lines = model calculations.

accounts for the sum of  $\text{COD}_I + \text{COD}_M$ . In this study, given the high biodegradability of the raw effluent and on the basis of data reported for similar effluents,<sup>12</sup>  $\text{COD}_I$  was considered to be absent in the raw wastewater. Thus, the calculation of biodegradation kinetic parameters was accomplished by considering the model of Dang and co-workers<sup>13</sup>

$$\frac{d(\text{COD}_B)}{dt} = -\frac{\mu}{Y_H} \frac{\text{COD}_B}{K_S + \text{COD}_B} X_A \quad (2)$$

$$\frac{d(\text{COD}_M)}{dt} = Y_M \frac{\mu}{Y_H} \frac{\text{COD}_B}{K_S + \text{COD}_B} X_A \quad (3)$$

$$\frac{d(X_A)}{dt} = \mu \frac{\text{COD}_B}{K_S + \text{COD}_B} X_A - b \times X_A \quad (4)$$

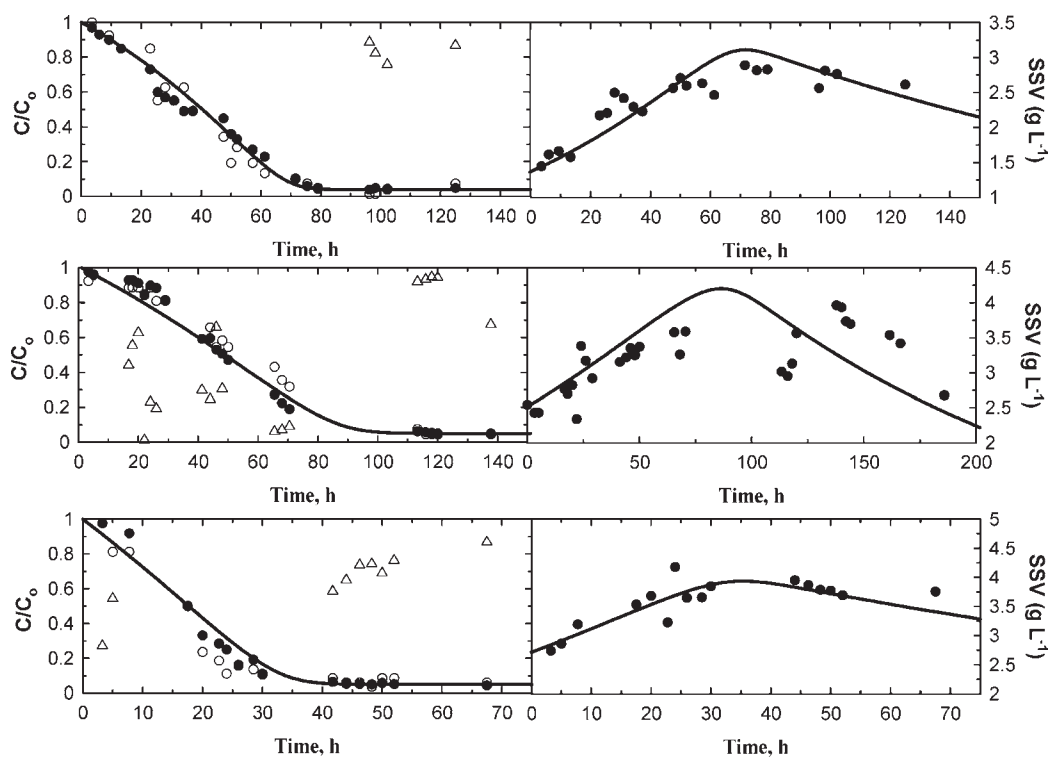
where  $\mu$  is the maximum specific growth rate ( $\text{h}^{-1}$ ),  $Y_H$  is the yield coefficient for heterotrophic bacteria,  $K_S$  is the half-saturation constant for growth ( $\text{mg L}^{-1}$ ),  $X_A$  is the active biomass concentration (measured as volatile suspended solids),  $Y_M$  is the soluble microbial product concentration coefficient, and  $b$  is the specific endogenous respiration rate ( $\text{h}^{-1}$ ).

Differential eqs 2–4 were numerically solved and the unknown parameters adjusted to fit experimental and calculated results. Obviously, the number of unknown constants is too high, and estimated parameters strongly depend on the initial guess in the optimization procedure. Consequently, common literature values were initially used in the fitting process. Figure 2 shows an acceptable modeling of COD and  $X_A$  by using the parameters listed in Table 2.

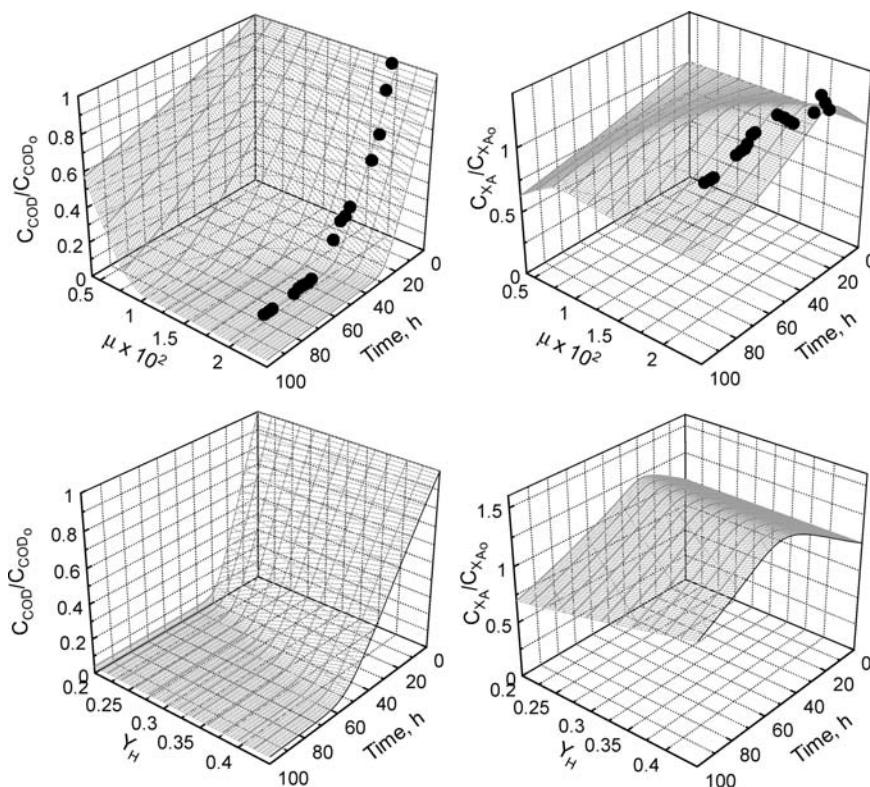
Additionally, for comparison purposes, Figures 3 and 4 display the results obtained when whey wastewater was first pretreated with FeCl<sub>3</sub> (FeCl<sub>3</sub>-PTted) or CaOH<sub>2</sub> (CaOH<sub>2</sub>-PTted).

From Figures 2–4 it is inferred that FeCl<sub>3</sub>-PTted and CaOH<sub>2</sub>-PTted wastewaters contain a COD more prone to biodegradation than COD from untreated effluents. In any case, given the differences in operating conditions, the previous statement could be corroborated by direct comparison of biological kinetic parameters (Table 2). Thus, the value of  $\mu$ , directly related to the COD degradation rate, is 4–5 times higher when pretreated wastewater is bioprocessed in comparison to the untreated effluent. Moreover,  $Y_H$ , the yield coefficient for heterotrophic bacteria, also decreases in value for pretreated wastewaters. This involves a faster COD removal and a lower sludge production. The influence of these two parameters ( $\mu$  and  $Y_H$ ) is illustrated in





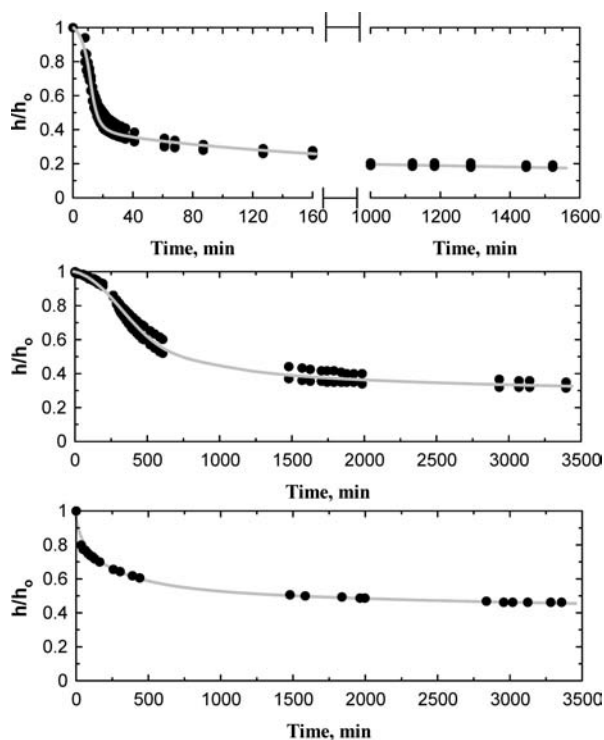
**Figure 4.** Aerobic biodegradation of  $\text{CaOH}_2$ -PTted wastewater. Experimental conditions at  $T = 290 \text{ K}$  and  $\text{pH } 7.5$ , COD ( $\text{mg L}^{-1}$ ), BOD ( $\text{mg L}^{-1}$ ), turbidity (NTU), and  $X_{A0}$  ( $\text{mg L}^{-1}$ ): (top) 8733, 6700, 14.8, and 1362; (middle) 9088, 7500, 90.2, and 2546; (bottom) 5400, 4500, 27.1, and 2714, respectively. Symbols: ●, COD ( $X_A$  in right-hand figures); ○, BOD; △, turbidity ( $T$ ) removal ( $1 - T/T_0$ ). Lines = model calculations.



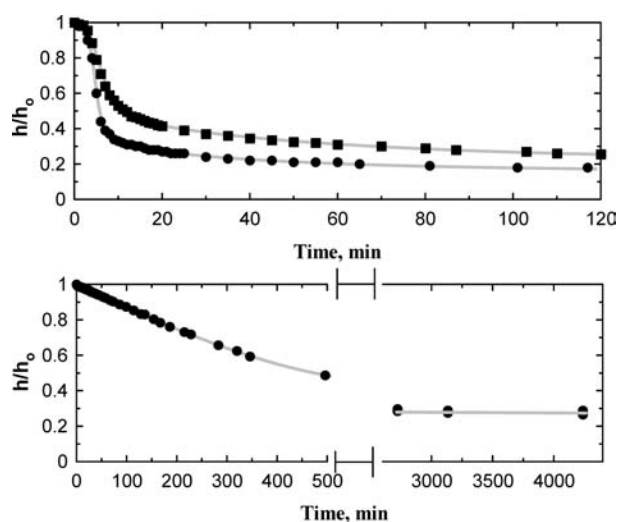
**Figure 5.** Aerobic biodegradation of wastewater. Influence of  $\mu$  and  $Y_H$  on process modeling (other parameters:  $K_S = 800$ ,  $Y_P = 0.04$ , and  $b = 0.008$ ). Experimental conditions: COD,  $6430 \text{ mg L}^{-1}$ ,  $X_{A0}$ ,  $2580 \text{ mg L}^{-1}$ . Symbols: ●, experimental data for NaOH-PTted wastewater.

Figure 5. Also, the experimental points of a NaOH-PTted run are given in the same figure. As inferred from Figure 5,  $\mu$  exerts a

significant influence on the COD removal rate. Hence, under identical operating conditions (given in Figure 5 caption) and



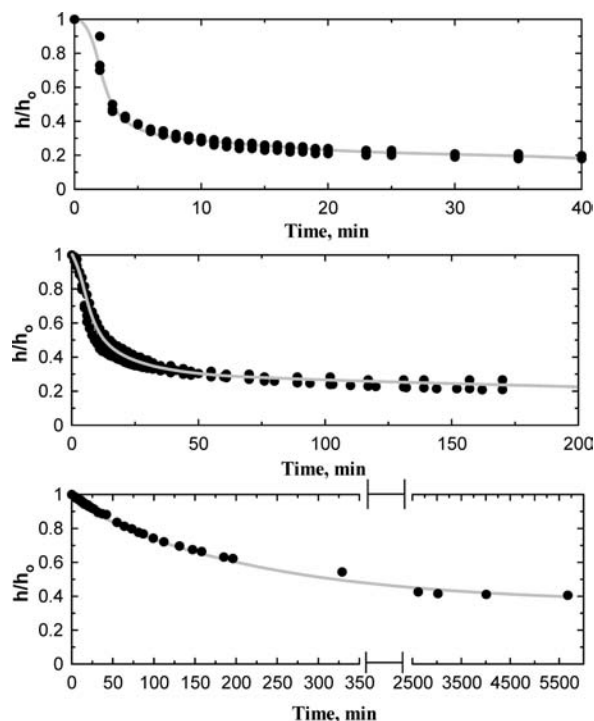
**Figure 6.** Solids sedimentation test after aerobic biodegradation of raw whey wastewater.  $X_{A0}$  concentration before the biological treatment:  $3.23 \text{ g L}^{-1}$  (top),  $6.53 \text{ g L}^{-1}$  (middle),  $8.51 \text{ g L}^{-1}$  (bottom).



**Figure 7.** Solids sedimentation test after the aerobic biodegradation of  $\text{FeCl}_3$ -PTted whey wastewater.  $X_{A0}$  concentration before the biological treatment:  $1.36 \text{ g L}^{-1}$  (top, circle symbols),  $2.45 \text{ g L}^{-1}$  (top, square symbols),  $4.79 \text{ g L}^{-1}$  (bottom).

similar values for the rest of the kinetic parameters, an increase of  $\mu$  from 0.004 to 0.02 involves a decrease in the COD half-life from 125 to 17.5 min.

A comparison of the kinetic parameter values obtained in this study and those reported by Orhon and co-workers<sup>12</sup> for a similar effluent reveals a lower  $\mu$  (bibliographic value of  $0.13 \text{ h}^{-1}$ ), higher  $K_S$  (bibliographic value of  $100 \text{ mg L}^{-1}$ ), and comparable values for the rest of the parameters.



**Figure 8.** Solids sedimentation test after the aerobic biodegradation of  $\text{CaOH}_2$ -PTted whey wastewater.  $X_{A0}$  concentration before the biological treatment:  $1.36 \text{ g L}^{-1}$  (top),  $2.55 \text{ g L}^{-1}$  (middle),  $6.50 \text{ g L}^{-1}$  (bottom).

*Settling Characteristics of the Biosludge.* Another important aspect to take into account in an activated sludge process is the settling capacity of microorganisms. Figures 6–8 show the solids interface profile for experiments conducted at different initial biomass concentration. As observed from these figures, no significant differences are observed in the sedimentability tests regardless of the wastewater processed (raw or pretreated). However, an unacceptable deterioration of the liquid–solid separation rate and extent is experienced when high concentrations of solids are initially present. Thus, values of roughly 80% of solids are removed in pretreated wastewaters when the lowest microorganism concentrations were used; however, increasing the initial amount of biosolids to values in the range of  $5\text{--}6 \text{ g L}^{-1}$  involves an increase in time in the interval of 300–500 min to achieve a poor 50% solids removal. Moreover, extended times do not significantly improve solids separation, experiencing final removal efficiencies in the proximity of 60–70%. According to these results the addition of a low amount of microorganisms is recommended.

Some preliminary sedimentation tank design calculations can be carried out by considering the most favorable cases (lowest initial  $X_A$  concentration) after the biological processing of pretreated wastewaters. Thus, the method of Coe–Clevenger was adopted for that purpose (see ref<sup>8</sup> for details). In the case of  $\text{FeCl}_3$ -PTted wastewater, if the initial  $X_A$  concentration is  $1.36 \text{ g L}^{-1}$  and the sludge from the sedimentation tank is concentrated 3.3 times (around  $4.5 \text{ g L}^{-1}$ ), the minimum clarifier area needed for a small plant (wastewater flow rate of  $100 \text{ m}^3 \text{ day}^{-1}$ ) would be approximately  $12 \text{ m}^2$ . If the same analysis is completed with the  $\text{CaOH}_2$ -PTted effluent, the area necessitated is reduced to roughly  $5 \text{ m}^2$ .

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