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Use of thermal coagulation, separation, and fermentation processes for dairy wastewater treatment

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ABSTRACT

Based on its large water consumption, dairy industry is considered as one among the most polluting food industries. The present work is related to investigations about water management practices in one Tunisian dairy plant where two effluents' generators were identified as the most polluting following their COD assessment. The first was the generated water from DECREAMING (D) machines washings with COD value of 112 g L^{-1}, and the second source is BACTOFUGATE (B) with COD value of 196 g L^{-1}. Thermal coagulation was performed for (D) and (B) samples leading to media clear phase separation. After decantation, recuperated supernatants were filtrated. The recorded COD removal amount exceeded 90% for both samples filtrates. Additional biological treatment was performed with mediums based on the obtained filtrates. Isolated *Candida* strains, *Lactobacillus* and baker's yeast strains were inoculated in batch process fermentation. Different biological treatment effects were evaluated using musts COD assessment at the end of fermentation after biomass removal. The reduction in organic load was important with the appropriate inoculated strains.

Keywords: Dairy wastewater; Treatment; Thermal coagulation; Separation; Fermentation

1. Introduction

The dairy industry involves processing raw milk into products including milk, butter, cheese and yoghurt, using processes such as chilling, pasteurization, and homogenization. Typical byproducts include buttermilk, whey, and their derivatives [1]. Large amounts of water are used during the process producing effluents containing dissolved sugars, proteins, fats, and possibly residues of additives.

According to Ali et al. dairy farm wastewater COD concentration varies in the range of 2–7 g L^{-1} depending on wastewater management, climate, operation conditions, and types of flushing [2]. The dairy industry wastewater is primarily generated from the

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cleaning and washing operations in milk processing plants. It is estimated that about 2% of the total processed milk is wasted into drains [3]. By the way, dairy wastewater contains large quantities of milk constituents such as casein, inorganic salts, besides detergents and sanitizers used for washing [4]. Therefore, a treatment/reuse system for dairy wastewater needs to be developed. The application of conventional methods (aerated lagoons, activated sludge processes, trickling, and rotating biological contactors) on dairy wastewater proves to be a difficult and complex process, which also consumes a great deal of energy at low efficiency rates [5].

Our work is focalized on one Tunisian bottling milk dairy industry (Centrale Laitière du Cap bon, CLC), where two effluents generating points have been identified as the most polluting through their COD values [6]. The first one is the water generated from DECREAMING machines washings with COD of about 112 g L^{-1}. The second source is the BACTOFUGATE with COD of about 196 g L^{-1}. It is important to remind that Bactofugation is a special form of separation in which specific types of micro-organisms are removed from milk and inactivated [7].

By joining the plant wastewater, those sources contribute largely towards their high organic matter to biochemical oxygen demand rise. Thus, its discharge in public sewer is strictly regulated by law and no longer accepted. The pressure of anti-pollution regulations requires that the dairy industry develops new technologies that can change the role of some effluents to valuable products [8]. Segregation of effluents from sanitary installations, processing, and cooling systems would facilitate the ability to recycle liquid rejects.

Previous works aimed to convert lactose, the major component of liquid wastes, to valuable products. The presence of lactose and other nutrients, essential for microbial growth, makes whey a potential raw material for the production of various bioproducts through biotechnological means [9]. Mawson showed that the bioconversion of milk whey lactose could reduce more than 75% of water pollution and generate products of interest for animal feed, human nutrition, and other alimentary, confectionery, pharmaceutical and agricultural companies [10]. Several methods have been proposed for whey valorization as major byproduct of the dairy industry [11–15]. In this respect, lactose-converting micro-organisms have been evaluated for the production of potable and fuel-grade alcohol [16–19], kefir-like whey drinks [20], and lactic acid [21,22]. Furthermore, micro-organisms production such as baking starter [23], probiotic starter cultures for fermented milk products [22,24,25], and cheese ripening [26,27] were investigated. The above research efforts for whey valorization resulted in significant organic load reduction. Consequently, scale-up processes were developed for potable and fuel-grade alcohol production [28] as well as for SCP and baker's yeast production from whey [29]. Kourkoutas et al. presented an economic evaluation of an industrial scale whey fermentation assays [30]. Another research regarded the expansion of ultrafiltration for milk pre-concentration, which results in important quantities of milk concentration permeate, a low-value byproduct of dairy industry. Seo et al. and Paseephol et al. have investigated lactulose production from this permeate using calcium carbonate-based catalysts [15,31].

In the frame of extensive efforts to reduce polluting load of dairy wastewater, an upstream hydric rejects segregation system for Decreaming machines washing water and Bactofugation separated milk was performed. The present research aims to investigate those dairy effluents thermal coagulation, separation, and serum fermentation treatment effects on dairy effluents COD removal.

2. Materials and methods

2.1. Sampling

Effluents were gained from a regional dairy industry (Centrale Laitière du Cap Bon) which is part of Delice Group, leader of the food industry in Tunisia in the dairy sector. The plant produces approximately 1,696,744 hL of milk drinks and three tons of butter per year [32]. According to the industry statistics, an average of 1,200 L of washing water is generated daily from Decreaming machines. An equal volume resulting from Bactofugation as separated milk is also recorded per day [6]. Sampling was performed once a month (during four months) at those process points considered as the most polluting sources. Decreaming washing water will be indicated as (D) sample and Bactofugation separated milk will be indicated as (B) sample in this paper. Analyzes were realized within 1 h of sample collection. Samples were stored at +2 to +6°C for 3 d.

2.2. Yeast isolation and identification

Ten milliliter of each raw sample was enriched in 90 mL of Sabouraud broth (Bio-rad, France) for 48 h at 30°C. A loopful of the medium was streaked on Chromagar *Candida* (Chromagar, France), and the different morphotypes obtained were cultured on Sabouraud Chloramphenicol agar (Bio-Rad, Marnes-La-Coquette, France) for 48 h at 30°C. Identification of *Candida* species was based on the following:

macroscopic characteristics of colonies, microscopic examination of yeast morphology (coloration), chlamydospores formation in Agar Tween (Difco, Paris, France), and colony color on Chromagar *Candida* (Chromagar, France). Strains were stored at 4˚C on Sabouraud dextrose broth (Bio-Rad) supplemented with glycerol at 10% (v/v). Biochemical characteristics were studied using carbohydrates assimilation test using both Api Candida and Api 20C systems (bio-Mérieux, Marcy l'Étoile, France).

Three *Candida* strains were isolated and identified from dairy effluent samples. They were cultivated on Sabouraud medium (30˚C for 48 h) to be inoculated in the fermentation medium. Commercial *Saccharomyces cerevisiae* strain was reactivated on Sabouraud broth (Hi Media), maintained in surface streaks on Sabouraud Glucose Agar (Hi Media) with Chloramphenicol plates. Strains were incubated for 24 h at 30˚C to be inoculated in 30 ml of seed culture containing (g L^{-1}): glucose, 20; KH_2PO_4, 2.4; $MgSO_4$, 0.2; urea, 2.4; yeast extract, 2.6 and grown at 30˚C on rotary shaker incubator at 150 rpm for 18 h.

Lactobacillus plantarum and *Lactobacillus paracasei* were cultivated on MRS broth medium at 37˚C for 18 h with 150 rpm agitation speed.

2.3. Samples treatment

Heat treatment was performed in glass vessels for (D) and (B) samples using heating mantle at different bearing temperatures (20, 40, 60, 80, and 100˚C). Clotting time, indicated as time from sample exposure to the desired treatment temperature to the formation of the first visible floccules, was measured. After thermal coagulation, samples were decanted in conic device for 24 h. Supernatant was recuperated for COD assessment. Obtained supernatant was passed through folded gauze placed into a glass funnel. The filtrate was recuperated for COD assessment.

2.4. Fermentation

After samples filtration, collected filtrates were used as fermentation mediums approached in two ways:

(1) A single-state fermentation using a pure yeast/*Lactobacillus* strain (e.g. [19,33,34]).
(2) A two-stage fermentation in which the lactose is first converted by a pure culture to lactic acid, which is consumed by the yeast strain (e.g. [35]). This approach allows yeast which cannot use lactose, such as *Saccharornyces cerevisiae* (bakers' yeast), to be produced [36,37].

No pH adjustment was done. Mediums were autoclaved (121˚C for 15 min). Aseptic inoculation was realized with micro-organism seed cultures prepared previously. Fermentations were performed in batch into Erlenmeyer flasks (250 mL) containing 50-mL working volume. The basal culture conditions are: fermentation time, 24 h as reported by [24]; temperature, 30˚C (yeast), 37˚C (*Lactobacillus*); inoculum 6% (v/v). The rotary speed of shaker incubator was fixed at 150 rpm. All experiments were carried in triplicate.

2.5. Analytical methods

The pH was measured at different temperatures during heating, at the beginning and at the end of fermentation, using a multiparameters device Consort C860. After separation, resulting supernatants and filtrates were recuperated for COD values determination using reflux method with opened system as reported by Rodier et al. [38]. At the end of fermentation, the culture was harvested by centrifugation (3,500 rpm during 15 min) to provide clear supernatant. COD assessment was performed for free biomass fermented musts.

3. Results and discussion

3.1. Strain isolation and identification

Three morphotypes were obtained on CHROMagar™*Candida* based on the color of the colonies: blue, white, and pink colonies. Three different colonies were purified on Sabouraud Chloramphenicol agar and identified as *Candida tropicalis*, *Candida lusitaniae,* and *Candida krusei* based on the morphological, microscopic, and biochemical characteristics obtained on API strips.

3.2. Thermal coagulation and pH variation

To recover the major component of dairy wastewater, it was obvious that heat treatment is efficient process with extreme rapid response. Preliminary tests confirmed that a short-time exposure of few minutes yields two phase formation. Heat treatment was performed for (B) and (D) samples and phase separation was settled during decantation. In fact, under heat effect micellar casein structure is destabilized, which causes mixture clotting and casein precipitation. The pH profiles under temperature effect are illustrated in Fig. 1.

No aggregates were noticed at 20 and 40˚C temperatures during experimentation for treated samples. According to (B) sample, a clotting time of 2 min at 60˚C was measured. Mixture pH was close to

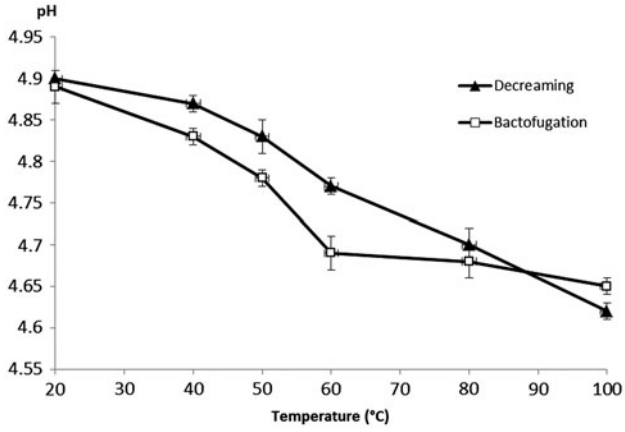

Fig. 1. pH variation during heat treatment of Decreaming sample (▲) and Bactofugation sample (■).

the casein isoelectric point (4.6). Casein micelles aggregate very fast and sediment, then clear phase separation was observed. However, for (D) sample, it was necessary to reach 100°C as treatment temperature to record a clotting time of 5 min when pH mixture was (4.62). Upon heat treatment of milk above 60°C, several processes take place and denaturation of whey proteins is the most obvious [39,40]. Meanwhile, no noticeable effects are observed on the casein micelle fraction during heat treatment between 70 and 100°C for ordinary milk [41]. Research on heat treatment of milk showed that the final composition of the whey proteins mixture and casein micelles depends on the pH and temperature of heat treatment [40,42,43]. So, it is important to mention that the pH of milk varies from 6.55 to 6.98 during a milking season [44,45]. And initial pH values of Decreaming washing waters (D) and Bactofugation effluents (B) were very close to 5 (4.9 and 4.89, respectively). This can probably accelerate casein aggregation under heat effect. Difference, in gelling temperature between (B) and (D) samples, was probably due to mediums' characteristics and microstructures. Decreaming washing waters are more diluted than Bactofugation effluents, and their composition is quite rich with fat than casein content. Researches where either changes in the milk composition were made or model systems were used have shown that salt concentration and composition, the ratio between casein micelles and whey proteins and total protein concentration are also determining denaturation kinetics [43,46–48].

3.3. Separation techniques

Decantation and filtration were applied as separation techniques for the mixtures after heat treatment. A simple observation of settling material showed that (B) sample coagulation resulted in a firm curd, which can be easily separated. However, (D) sample coagulation resulted in suspended aggregates. Supernatants and filtrates were recuperated for COD assessment. Results are resumed in Table 1.

According to Decreaming samples, COD values pass from 112 to 43.6 g L^{-1} after decantation, leading to 61% COD removal amount. With Bactofugation samples, the COD removal amount after decantation was more important (82%). A COD values shift from 196 to 33.6 g L^{-1} was recorded. Decantation impact was more interesting with Bactofugation sample (B) due to the medium texture allowing an easy phase separation by decantation after exhibition to a moderate heat treatment (60°C). On the other side, Decreaming sample (D) presented dispersed different sized aggregates making decantation more or less slow and inefficient.

In order to ameliorate the recuperated liquids quality, supernatants filtration was performed as described in Section 2.3. Two repetitions were carried out for each sample. Table 1 presents COD determination values. It is clear from those results that filtration technique had good effect on organic load decrease. Filtrates COD values reached, respectively, 11.1 and 3.3 g L^{-1} for (B) and (D) samples. Thereby, the recorded COD removal amount exceeded 90% for both samples. It is important to mention, that even if filtration process generates better effluent quality, it is obvious to meet clogging issues. So, it is advised to proceed always with decantation at first. Besides, a vacuum filtration system may moderate such issue.

In the transformation cycle, industrial wastewaters are made mainly by cream/butter washing water. In general, the first washings of butter production are separated. Because of their fat and nutrients content, they are well-suited products for livestock feed [49]. Thus, the recuperated solid matter resulting from thermal treatment and separation technics was dried until fixed weight at 60°C and passed for nutriments analyses to investigate its performance as livestock feed fraction.

Table 1
Decreaming and Bactofugation samples COD (g L^{-1}) assessment after decantation and filtration

COD (g L^{-1})	Initial	Supernatant	Filtrate
Sample (D)	112 ± 2.8	43.6 ± 1.1	11.1 ± 0.4
Sample (B)	196 ± 3.1	33.6 ± 0.6	3.3 ± 0.3

3.4. Effect of COD fluctuation in the process inlet

The use of thermal coagulation with separation processes for dairy effluents treatment was explored for a longer time period. The effect of COD fluctuations in the process inlet on the system operation was investigated during four months where samples were withdrawn periodically. Fig. 2(a) and (b) shows the (B) and (D) samples COD fluctuation, respectively; during the months of March, April, May, and June in the process inlet and its effect on thermal coagulation and separation processes efficiency to decrease residual COD.

Inlet COD values range from 112 to 204 g L^{-1} for (D) samples and from 188 to 236 g L^{-1} for (B) samples. It is noticeable that both COD profiles have increasing trend from the month of March to June. This might be closely associated with the processed milk in each period of production, since its quality varies according to milking season, climate, and operation conditions [2,50]. Besides, seasonal temperatures affect effluents' characteristics [50].

In regard to the used system effect, the global-recorded COD removal rates for both (D) and (B) samples exceed 82% for all the test period. Thus, the contribution of thermal coagulation process was more important for both (D) and (B) samples than filtration. The average contribution in COD removal by thermal coagulation process was about 75% for Bactofugate samples and only 58% for Decreaming samples. Yet, the average contribution in COD removal by filtration technique was more considerable for Decreaming samples (29%) than for Bactofugate samples (14%). The use of thermal coagulation and separation techniques could ensure high removal rates although the inlet COD fluctuations.

Practically, the technico-economical analysis based on the performance of the proposed system would be difficult to estimate since the presented results are lab-scale data. The feasibility of thermal coagulation for dairy effluents in this process might be achieved using steam excess under high temperature (60–100°C) generated from steam boiler within the dairy industry itself.

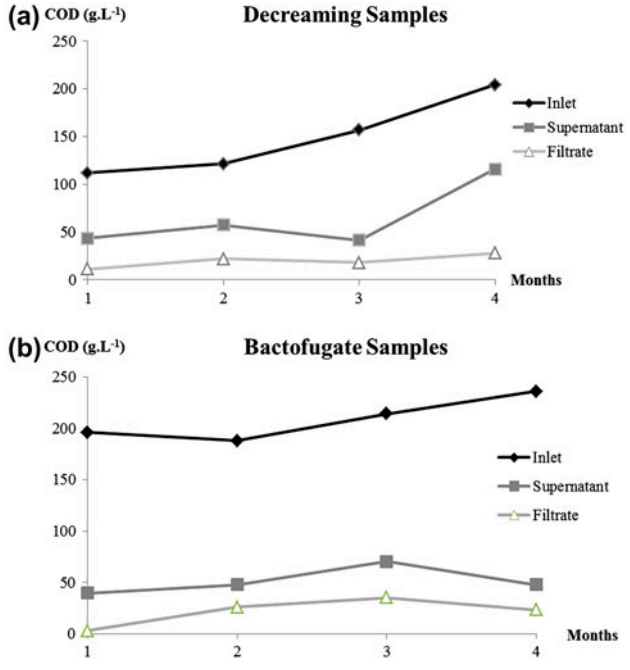

Fig. 2. (a,b) Inlet COD fluctuation of Decreaming samples (a) and Bactofugate samples (b) during 4 months and its effect on thermal coagulation and separation processes efficiency.

3.5. Biological treatment: fermentation

Commonly, fermentation of raw dairy wastewater is performed under anaerobic process. In fact, raw samples having high COD values would be fermented using methanogen bacteria. However, such process will generate important biomass with CH_4 and CO_2 gases emission [51]. Therefore, thermal pre-treatment of raw dairy wastewater was necessary not only to reduce residual COD values for bacterial fermentation, but also to recuperate mainly milk proteins from dairy effluents. Furthermore, the major component of the obtained wheys was particularly carbohydrates. In order to make as lower as possible (D) and (B) effluents organic load, fermentation was performed. Only yeast strains like *Kluyveromyces*, *Candida,* and *Saccharomyces* seem to be grown commercially, although the production of a number of other organisms has been considered [10].

3.5.1. One-stage fermentation by pure Candida strains

Biological treatment results with different inoculated strains are resumed in Fig. 3.

For the culture medium based on (B) sample filtrates, a COD removal rate of 95% was recorded with *C. tropicalis* fermentation and 78% with both *C. lusitaniae* and *C. krusei* strains. However, for the culture medium based on (D) sample filtrate, *C. tropicalis* strain has given an important COD removal rate of 82% as compared to *C. lusitaniae* and *C. krusei* strains inoculated in the same medium where COD removal rate did not exceed 45%. This may be interpreted by a

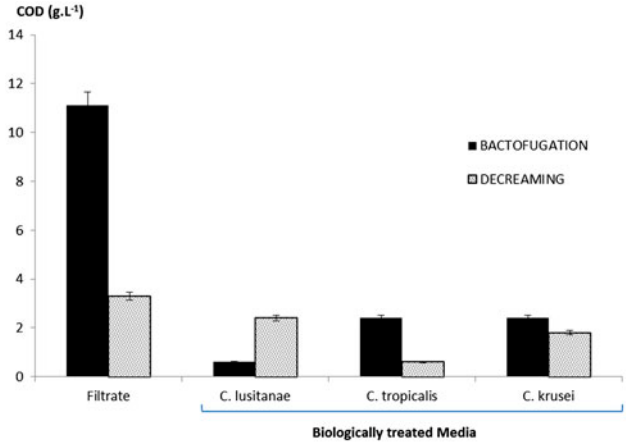

Fig. 3. Effect of biological treatment by *C. lusitaniae*, *C. tropicalis*, and *C. krusei* fermentation of Decreaming (D) and Bactofugation (B) filtrates on COD (mg L^{-1}) abatement.

better adaptation of the *C. tropicalis* strains to the medium based on (D) filtrate.

3.5.2. *One stage fermentation by pure* Lactobacillus *strains*

Biological treatment results with *Lactobacillus* sp. inoculated strains are resumed in Table 2. It seems that micro-organisms act differently in regard to COD abatement in culture mediums.

The COD removal exceeded 80% with both *L. plantarum* and *L. paracasei* culture on Decreaming filtrate. Meanwhile, COD removal with both *Lactobacillus* strains culture did not exceed 27% on Bactofugation filtrate. But, it is important to mention that final COD values were limited to 2.6 and 2.2 g L^{-1}. Considering dilution effect by joining the industry wastewater, such values are considered relatively acceptable according to the discharge standards in the Tunisian National Sanitation Utility (ONAS) sewages fixed at 1 g L^{-1} (Tunisian Standards NT 106 002).

3.5.3. *Two-stage fermentation by* L. plantarum *and* S. cerevisiae *strains*

In the two-stage fermentation process, lactose was first converted using a pure *L. plantarum* culture to lactic acid, which was consumed later by *S. cerevisiae* strain which cannot utilize lactose. After culture musts centrifugation, the obtained effluent COD values were 2.88 g L^{-1} for (D) sample, but only 0.4 g L^{-1} for (B) sample (Table 2). By the way, this combined strains process seems to be not beneficial for (D) sample treatment comparing with pure *L. plantarum* culture effluent. However, this process led to an important organic removal rate increase for (B) sample (87%).

Batch processes for biomass production from dairy effluents have been developed in this study. Culture media supplementation with additional nitrogen and other nutrients may promote optimal yeast growth [52]. The supply of adequate oxygen for bacterial growth is a major process consideration to get better COD removal rates. Otherwise, inefficient aeration would lead to ethanol production which will increase the effluents organic load [53]. In fact, in case of oxygen deficiency, the production of ethanol and its accumulation in the medium is due to the use of a substrate portion for the formation of secondary metabolites at the expense of the energy required for biomass synthesis. Therefore, ethanol accumulation in the fermentation medium contributes to biomass free effluent COD increase. The potential of lactose bioconversion to produce useful products whilst reducing the COD of the incoming matrix by at least 70–80% has been demonstrated [10]. But in case of treatments combination, the overall reduction in oxygen demand typically exceeds 95% [10]. The optimal treatment combinations in this study were:

Table 2
COD values (g L^{-1}) of the effluents generated by fermentation operations and resulting abatement rates (%)

Fermentations		Sample	COD (g L^{-1})	Abatement rate (%)
Filtrates		(B)	3.3 ± 0.30	–
		(D)	11.1 ± 0.40	–
Inoculated strains	*L. plantarum*	(B)	2.4 ± 0.23	27
		(D)	1.92 ± 0.11	82
	L. paracasei	(B)	2.6 ± 0.25	21
		(D)	2.2 ± 0.20	89
	L. plantarum + *S. cerevisiae*	(B)	0.4 ± 0.12	87
		(D)	2.88 ± 0.20	74

(1) Heat treatment (100˚C), filtration followed by *C. tropicalis* fermentation for (D) effluent.
(2) Heat treatment (60˚C), filtration followed by two-stage fermentation using *L. plantarum* and *S. cerevisiae* for (B) effluent.

The proposed treatment system achieved overall COD removal rates exceeding 99%.

4. Conclusion

Water management practices in CLC dairy plant show that effluents organic load is mainly coming from two polluting origins: the first one is the water generated from DECREAMING machines washings with COD of 112 g L^{-1}, and the second source is BACTOFUGATION separated milk with COD of 196 g L^{-1}.

Coupled thermal and biological treatment seems to be a promising way for the reduction in organic pollutants discharged from industrial dairy sources. Significant COD reduction at variable organic loadings was recorded with tested treatment combinations during this study. Thermal coagulation with filtration technique leads to COD removal amount of 98.4% for (B) sample and 90% for (D) sample. Biological treatment with suitable strain inoculation in the obtained filtrate has limited COD value at 0.6 g L^{-1} with *C. lusitaniae* on (B) filtrate and *C. tropicalis* on (D) filtrate. The two-stage fermentation process using *L. plantarum* and *S. cerevisiae* enabled to reach a lower COD value (0.4 g L^{-1}) for (B) sample. Those values (0.6 and 0.4 g L^{-1}) are in perfect conformity with Tunisian standards fixed by ONAS (1 g L^{-1}). Furthermore, it is important to remind that this treatment was done on two source points. By joining plant hydric effluents, COD values will be much lower undergoing dilution effect.

Symbols

BOD	—	biological oxygen demand
CLC	—	Centrale Laitière du Cap Bon
COD	—	chemical oxygen demand
ONAS	—	Office National d'Assainissement Sanitaire
SCP	—	single cell protein
rpm	—	rotation per minute

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