Journal of Applied Science and Environmental Studies
JASES
http://revues.imist.ma/index.php?journal=jases

Physico-chemical and biological treatment of a dairy liquid effluent.

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Received 29 Nov 2018 ; Revised 12 Jan 2019, Accepted 17 Jan 2019
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Keywords
Dairy effluent, pollution, physico-chemical treatment, biological treatment.

Abstract
The effluent dairy industries are among the most organic-rich food waste. With high values of lactose, proteins, vitamins and minerals, it is a favorable medium for the growth of microorganisms such as fecal coliforms and streptococci. In addition, it has high levels of COD, total nitrogen and total phosphorus that indicates a significant pollution of the ecosystems where it is drained. This study focuses on the treatment of dairy effluent by two different methods. The first is a physicochemical treatment by coagulation, flocculation; using aluminum sulphate and sodium alginate. While the second process is a biological treatment using *Pseudomonas fluorescens* and *Bacillus spp*. The results show a decrease of about 30% of chemical oxygen demand, 49% of turbidity, 78% of suspended solids and 20% of the total phosphorus. The second process has shown a significant reduction of all parameters better than the physico-chemical treatment, with fluctuations in efficacy between the strains tested. In conclusion, we have developed biotechnological processes, which are simple, economical and environmentally friendly for treating the discharges of the dairy industry and reduce polluting factors.

1. Introduction

Morocco's milk production doubled in ten years from 1.25 billion liters in 2007 to 2.5 billion in 2017 according to the Ministry of Agriculture and Rural Development. In parallel, there is a production of discharges more and more rich in oragnic and mineral by-products, generating a real environmental problem.
The environmental problems of the dairy industry are manifested by pH variability of the discharged liquid waste and its richness in organic matter and bacteriological. Indeed, different types of liquid waste are produced; we find wastewater from cleaning equipment and pipes, water from cooling, domestic sewage, acidic and sweet whey [1, 2]. Whey forms the most polluting effluent waste from the dairy industry by its saturated biochemical composition organic (lactose,

proteins, amino acids such as lysine and tryptophan, group B vitamins such as thiamine and riboflavin in addition to microorganisms). However, it is of undeniable importance and can be valued at high added value for industrialists [3, 4, 5, 6].

In addition, the importance of managing these liquid effluents continues to grow as organic waste causes physicochemical and biological degradation of the ecosystem aquatic life (BOD greater than 5000 mg / L). Often, they cause excessive growth in algae and bacteria that consume more oxygen from the water leading to the disappearance progressive flora and fauna [6, 7].

Currently, environmental standards require manufacturers the establishment of an environmental management system, which allows respect for of the natural environment by controlling the composition of the rejects and by studying its influence on the fauna and flora of the environment. Several effluent treatment techniques are used. The choice between these techniques depends essentially on the characteristics of the effluent and the nature of the receiving environment [5, 6]. However, in Morocco, in most case, these effluents are not treated and are discharged directly into the aquatic environment [1, 7].

The first objective of this study is to carry out a qualitative and quantitative characterization of the dairy effluent of a large dairy industry in Morocco. The second objective is to treat these effluents with two different processes. The first is a physicochemical treatment by coagulation-flocculation using aluminum sulphate and alginate sodium and the second is a biological treatment that using microorganisms in suspension; *Pseudomonas fluorescens* and *Bacillus spp*. In addition, different parameters and factors are optimized to increase the effectiveness of treatment and to have the most efficient processes to ensure the best results.

2. Experimantal details

2.1. Substrates

The substrate has been obtained from rejection of the large dairy industry during several periods of the year 2011. The samples were taken from sterile polyethylene vials and transported to the laboratory in less than an hour. They were mixed and kept at 4°C until use. Conservation has been made according to the general guide for the conservation and handling of samples (ISO 5667/3).

2.2. Characterization of whey

The physicochemical analysis of the effluent was carried out by methods described according to ISO 5667/3 standards [1]. The analyzes concerned; temperature, pH, conductivity, suspended solids (MES), chemical oxygen demand (COD), biological oxygen demand (BOD 5), nitrogen (nitrite, nitrate, Kjeldhal nitrogen) and phosphorus compounds (total phosphorus).

For bacteriological analyzes, we were interested in the enumeration of fecal streptococci on agar plates incubated at 37 °C and the enumeration of fecal coliforms by the most probable number method after culture on lactose broth incubated at 44.5 °C [1, 8].

2.3. Coagulation and flocculation of the effluent

Various coagulation and flocculation tests were carried out on the effluent in using a "test jar" according to the following principle; in a series of beakers containing the dairy effluent, different concentrations of the coagulant agent (sulfate of aluminum) with stirring for a short time (200 rpm) and at room temperature (24 ± 2 ° C). Then the flocculant was added to previous substrate (2.5 mg/l sodium alginate) with slight stirring (40 rpm for 20 min). The effectiveness of this treatment has been evaluated analytically by turbidity monitoring (measured in FTU: formazin turbidity unit), MES, COD, total nitrogen, total phosphorus and nitrate.

Table 1: Different concentrations of aluminum sulphate in the "Jar test"

Beaker	1	2	3	4	5	6	7
Concentrations of aluminum sulphate (mg/L)	0.5	1	2	4	6	8	10

2.4. Biological treatment of the effluent

This treatment was performed by inoculation of a sample of 500 ml of whey with *Bacillus spp*. or *Pseudomonas fluorescens* or with two bacteria at 10^9 UFC/ml. This association is used to study the effectiveness of both bacteria in the treatment and degradation of whey. These samples were placed in the shaker for 20 days at 100 rpm at room temperature (24 ± 2 ° C). 500 ml of uninoculated whey was prepared as negative control. All samples were oxygenated once a week. [6, 9, 10, 11]. The effectiveness of Biological treatment and its monitoring are studied by measuring absorbance, COD, SS, total nitrogen, total phosphorus and nitrate.

2.5. Analysis of effluent parameters

To determine the impact of releases and confirm the feasibility and the effectiveness of the physico-chemical and biological treatments carried out, the composition of effluent, pH, COD, BOD5 and COD/BOD5 ratio before and after treatments were sought according to different techniques [1, 9, 10].

3. Results and discussion

3.1. Characterization of the effluent

The dairy production of the studied industry has two periods; high lactation (March to September) and low lactation (October to February). The average annual volume of liquid waste discharged is 900 m3/d (including 7.5 m^3 of whey) with 4.7 liters of liquid effluent/liter of treated milk (Table 2).

Table 2: degree of discharge pollution from the processing industry.

Type of effluent	pH	Volume produced /L	COD (g/l)	DCO/DBO5
White water	5,5	3 à 4	2,5	1,3
Whey	4,3	0,75	6	1.5
Total effluent	4,5	4 à 5	11	1,7

In this work, several characteristics of effluent pollution were studied. Table 2 summarizes the parameters for estimating the degree of pollution of this waste. The ratio COD/BOD 5 is less than 2, more this ratio is reduced, more organic matter present in the discharge will be easily degraded by bacteria. More, the pH of all components is acid. The analyzes of the substrate sought reveal that the total effluent consists essentially of water, lactose, proteins, minerals and fat. The results are shown in table 3.

Table 3: Release Composition of the Processing Industry.

Constituents	Whey	Total effluent
Water (%)	93	95
Temperature (°C)	40	48
Flow (l/s)	13	13
pH	4,5	6,6
Turbidity (NTU×10)	115	144
Dry matter (g/kg)	62	102

Lactose (g/100g MS)	240	240
Protein (g/kg MS)	82,5	107
Minerals-ash (g/kg MS)	41	43
Total nitrogen Kjeldahl (g/l)	0,44	1,3
Total phosphorus (g/l)	0,149	0,225
Nitrates (g/l)	0,32	0,5
MES (g/l)	2,850	3
Organic acids (g/l)	11	12
Fat (g/l)	2,1	16
Fecal coliforms (CFU/ml)	-	2,08 × 105
Fecal Streptococci (CFU/ml)	-	3,11 × 105

Tables 2 and 3 present the physico-chemical characteristics and bacteriological total effluent. Recorded values of TSS, COD, BOD 5, total Kjeldahl nitrogen and total phosphorus as well as pH values and temperature far exceed the values set by Moroccan standards [12]. Also, the average bacterial load is 2.08 × 10^5 CFU/ml for fecal coliforms and 3.11×10^5 CFU/ml for fecal streptococci. The CF/SF ratio is less than 1, which indicates a fecal contamination of animal origin (fecal streptococci) of the samples studied.

3.2. Physicochemical treatment

The results of the physico-chemical treatment (coagulation-flocculation) by aluminum sulphate and sodium alginate are shown in table 4.

Table 4: Liquid milk rejection composition before and after treatment with coagulation – decantation.

Parameters analyzed	Values before treatment	Values after treatment
pH	4.5 adjusted at 6.5	7.1
MES g/l	3	0.9
Total COD g / l	11	7.33
Total BOD mg / l	6.4	2.1
Turbidity (NTU × 10)	144	70
Nitrates (g / l)	0.5	0.025
N-NTK g/l	1.3	0.22
P-PT g/l	0.225	0.05

This table shows a reduction of about 30% in the chemical oxygen demand at a concentration of 6 mg/l of aluminum sulphate (figure 1), 33% of the biological oxygen demand, 49% turbidity, 78% suspended solids (MES), 20% of total phosphorus, 5% of nitrates and 16.4% of total nitrogen.

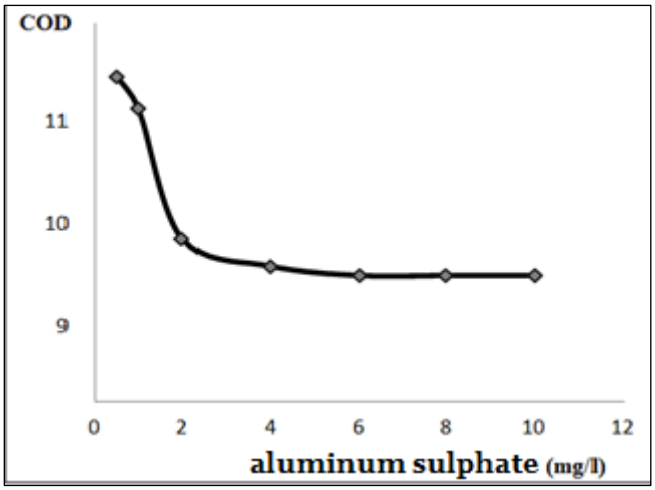

Fig. 1 : Effect of aluminum sulphate concentration (physicochemical treatment) on COD

Figure 1 shows the evolution of COD as a function of different concentrations of aluminum sulphate. In a first observation we noticed the appearance of jaws in beakers 4, 5 and 6, which have become turbid with time, probably because of the presence of micro focus.

3.3. Biological treatment

The growth of bacteria on the dairy effluent is a good indicator that this effluent is rich in nutrients. The following curves show the growth of *Bacillus spp*. and *Pseudomonas fluorescens* on whey (Fig 2 and 3).

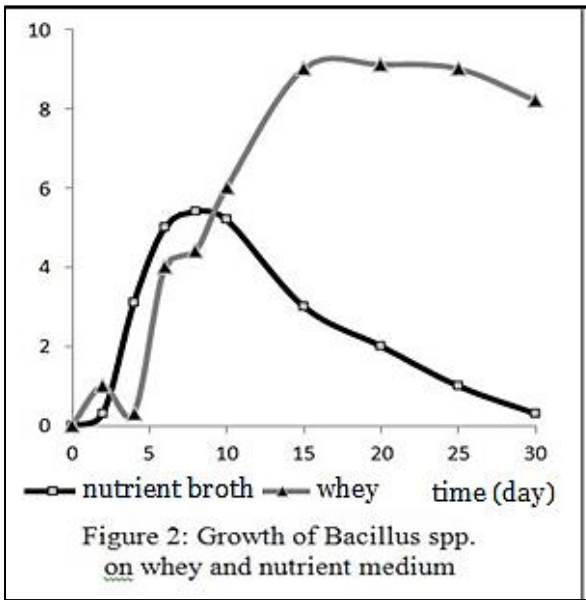

Figure 2: Growth of Bacillus spp. on whey and nutrient medium

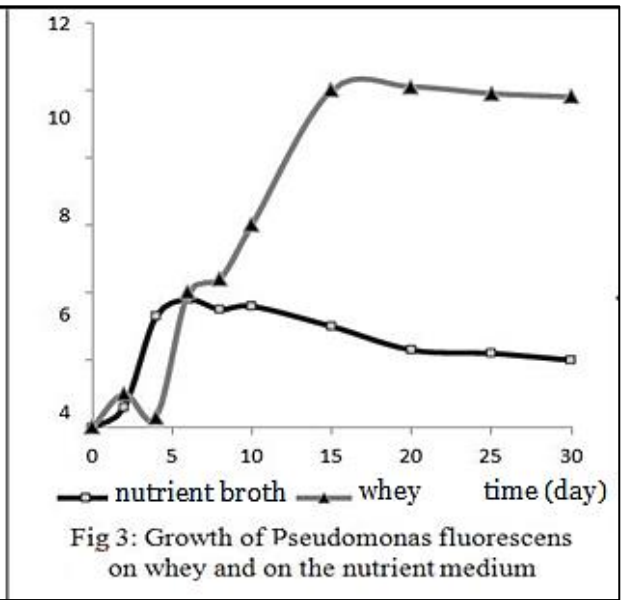

Fig 3: Growth of Pseudomonas fluorescens on whey and on the nutrient medium

We notice that both bacteria grow on whey during 15 days and continue to live beyond this period unlike bacteria grown on a nutrient medium that deteriorate and die after 15 days of incubation. This could indicate that whey is a favorable medium for the growth of two bacteria. In this work, we sought the effectiveness of biological treatment by *Bacillus spp*. and *Pseudomonas fluorescens* and then we compared it with the physicochemical treatment. The following figure shows the variation of COD depending biological treatment.

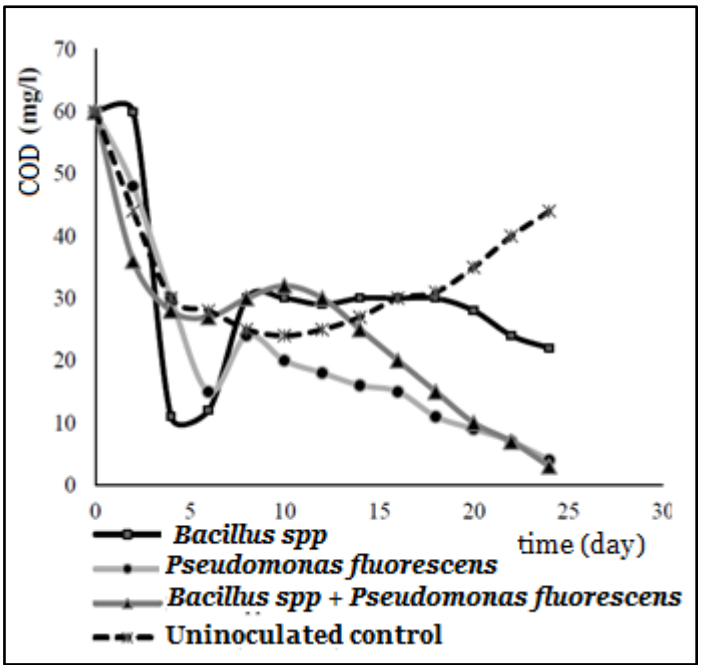

Fig. 4: DCO variation of whey as a function of biological treatment by *Bacillus spp*. and *Pseudomonas fluorescens*.

This graph shows a COD reduction of up to 92% during treatment *Pseudomonas fluorescens*, and about 86% with *Bacillus spp*. and *Pseudomonas fluorescens* for 24 days of treatment. While in the same conditions, *Bacillus spp*. allowed only a 54% reduction in COD. It indicates that the biological treatment of whey by *Pseudomonas fluorescens* is not only more effective than treatment with *Bacillus spp* but also more important as chemical treatment by flocculation and coagulation. On the other hand, the analyzes physicochemicals sought after biological treatment with *Pseudomonas fluorescens* indicate the reduction of 94% of total nitrogen and about 80% of nitrates.

Discussion

The physicochemical composition sought in this work (MES, COD, BOD 5, N-NTK and P-PT etc.) are well above international standards for limit values for liquid discharges [12]. The DOC values are higher than the value allowed by the Moroccan standard (0.5 g / l), but which remains lower than the COD of the oil extraction and lignocellulosic material processing industry [13, 14]. The recorded limit values of the BOD 5 far exceed the allowed value (0.1 g/l) and the ratio COD/BOD5 is greater than 0.3 which implies biodegradability [15]. These results can be explained by the presence in milk effluents, in addition to fermentation microorganisms, of multiple cleaning products (detergents and various additives as well as disinfection by sodium hypochlorites and the use of soda) used in the various operations of transformation. The average annual volume of waste released is also slightly elevated than that mentioned in the literature [16]. It would therefore be necessary to reduce wastewater discharges to within 1.5 and 2 liters of effluent per liter of treated milk also to recycle and reuse non-treated water polluted like cooling waters and condensates [1, 3].

Flocculation and coagulation are used to remove fat, oil, phosphorus, suspended solids (MES), heavy metals, and others. It is recognized that pH influences the abatement rates of pollution contained in wastewater [17]. For our study, the optimal pHs for removal of suspended matter, chemical oxygen demand, Kjeldahl total nitrogen and total phosphorus range from 5.5 to 7 for coagulum at aluminum base. Agitation during physicochemical treatment ensures good distribution of coagulants and chemical destabilization of colloids, then it facilitates the contact between the particles and avoids damaging the formed flocs [18]. So, the use of a coagulation-flocculation pretreatment makes it possible to reduce COD, which is the biggest problem in

effluent discharge. This efficacy can be increased by use of electrocoagulation, a method that could not be tested in our laboratory. This method allows the reduction of the chemical and biochemical oxygen demand (COD, BOD 5) and the reduction of microorganisms [1, 3, 16]. Aluminum sulphate is considered to be the most potent coagulant reduces the high load of COD phosphate and total nitrogen from wastewater and oil mill effluents [13]. Even without adjusting the pH of the medium, the Aluminum sulphate can easily coagulate at a whey pH or total effluent [10, 19]. Also, we used sodium alginate because it is very effective as a flocculant [1, 17]. Combination of flocculation and coagulation by the aluminum sulphate and sodium alginate together provide good results [10].

We chose the biological suspension treatment because it exposes several advantages, indeed, it presents an availability of microorganisms, a clean technology, economical treatment (inexpensive, less maintenance and low demand energy), easy handling and applicable in laboratories [2, 5]. The main microorganisms used to treat effluents are usually lactic acid bacteria (Lactobacillus), phototrophic bacteria (Aspergillus and Galactomyces) and yeasts (Saccharomyces) [2, 20, 21]. In our study, the biodegradation of whey by *Bacillus spp*. and *Pseudomonas fluorescens* gave good results. We chose these two bacteria because they are easily encountered in biological labs and they are very prevalent in the environment they live mainly in water and wet soils at 24 °C to 35 °C and a pH ranging from 5 to 7, also and essentially they are not pathogenic [3].

In conclusion, a series of treatments to reduce the impact pollutant dairy effluents on aquatic and terrestrial ecosystems. So, the treatment by coagulation flocculation and biological treatment have allowed to reduce the organic, nitrogen and phosphorus load. The best result is obtained with the biological treatment that has guaranteed the respect of Moroccan standards. This process has the deserves to be at the same time depolluting because it reduces enormously the quantity of waste biological and chemical so it is simple, easy, feasible and economical to scale industrial

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