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Characterization, Physicochemical and Biological Treatment of Sweet Whey (Major Pollutant in Dairy Effluent)

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Abstract. The sweet whey resulting from cheese production is the most polluting among all types of dairy waste waters given that it contains a huge quantity of organic biodegradable matters. In this study, we realize two treatments of sweet whey; the first, a physicochemical one, by coagulation-flocculation using aluminum sulphate as a coagulant and sodium alginate as flocculent. The second is a biological treatment in suspension, using *Pseudomonas fluorescens* and *Bacillus spp.*

The physicochemical treatment by coagulation-flocculation has reduced 33 % of the chemical oxygen demand, 45% of the turbidity, 72 % of suspended matter and 20% of total phosphorus.

The biological treatment has reduced considerably all the studied parameters; the organic charge determined by the chemical oxygen demand COD has received a very significant reduction up to 90% using *Pseudomonas fluorescens* during 20 days. On the other side, the biological treatment realized with *Bacillus spp.* was less effective, reduced the COD with 54% under the same conditions of the treatment by *Pseudomonas fluorescens* and for the same period. But these two bacteria are equally effective in reducing the total nitrogen TN (92% reduction of TN during 20 days of treatment).

This work presents a biotechnology for the treatment of sweet whey. It's an economic method, simple and friendly environmental.

Keywords: sweet whey, physicochemical treatment, biological treatment, chemical oxygen demand, *Bacillus spp.*, *Pseudomonas fluorescens.*

1. Introduction

People in the Middle East in general and Lebanon in particular, are major consumers of small ruminants, which account for 25% of milk production in the country. The annual production of cheese in Lebanon is 21091t/year. [1]

The importance of the problem of liquid waste management continues to grow in Lebanon especially in the dairy industries that generate a huge organic pollution which can cause physico-chemical and biological degradation of the aquatic ecosystem by the presence of undesirable substances (nitrogen, phosphorus, carbon materials, microorganisms). [8]

These dairy industries generate different types of waste including: wastewater from the production line (cleaning of equipment and pipes) cooling water, domestic wastewater, the acid whey and sweet. The sweet whey form the most polluting effluent by its biochemical composition rich in organic matter (lactose, protein, phosphorus, nitrates, nitrogen) and is from 60 to 80 times more polluting than domestic sewage. [4]

Type of effluent	pH	Volume per liter of milk	Chemical Oxygen Demand COD(g/L)	Chemical Oxygen Demand/Biological Oxygen Demand COD/DBO_5	Total Nitrogen Total N (g/L)	Total Suspended Solid TSS (g/L)	Total Phosphorus Total P (g/L)	Fat (g/L)

Cheese Whey pressed	6	0.9	80 to 90	1.5	0.6 to 1	8 to 11	-	0.5

Fig. 1: Characteristics of sweet whey [6]

The main environmental problems related to milk production affect the pollution of water, air and biodiversity. They often cause a growth of algae and bacteria that consume oxygen in the water and eventually suffocate the rivers leading to the gradual disappearance of fish. Hence the need to treat dairy effluents by various processes: physicochemical (coagulation, flocculation and sedimentation) and biological (by adding microorganisms in suspension) [4]. In Lebanon and in the most cases, these effluents are not treated and are discharged into the aquatic environment.

This project therefore aims to use two types of treatment (Physico-chemical and biological), to track and monitor the effectiveness of reducing various parameters of pollution by various tests to optimize a new treatment method.

Parameters	Discharge standards	Conditions
COD (mg/L)	• 200 • 100	• If the maximum permitted daily flow does not exceed 100kg/d • Beyond 100 kg/j
TSS (mg/L)	50	-
Total N (mg/L)	30 in monthly average concentration	When the maximum daily flow is equal to or greater than 50kg/day
Total P (mg/L)	10 in monthly average concentration	When the maximum permitted daily flow is equal to or greater than 15 kg/day

Fig. 2: Discharge standards acceptable to reject dairy effluents [7]

Flocculation and Coagulation are used to remove grease, oil, phosphorus, suspended solids (TSS), heavy metals, etc… This method allows the reduction of the chemical and biological oxygen demand (COD, BOD) and the reduction in bacterial populations. Aluminum sulphate $Al_2(SO_4)_3$ was the coagulant used because it reduce the hardness and the load of phosphate in the wastewater[10] , It is better than the ferric chloride $FeCl_3$ in the reduction of COD, TN and TP, no need to adjust the pH [9]. It can easily coagulate at pH close to neutral which is the pH of sweet whey. We used also sodium alginate because it is an effective flocculent without modification [13]. It provides with $Al_2(SO_4)_3$ good results [10].The amount of coagulant and flocculent to use and the effectiveness of this process are dependent on pH, alkalinity and phosphate concentration. [3]

We chose the biological treatment in suspension as it has several advantages: availability and cheap construction, economic (inexpensive, less maintenance cost, low energy demand), easy handling, applicable in laboratory. That's why our study concerns the biodegradation of sweet whey. The main microorganisms used in the literature to treat the effluent are: lactic acid bacteria (*Lactobacillus* and *Pediococcus*) yeast (*Saccharomyces*) and phototrophic bacteria [12], *Aspergillus niger* and *Mucor hie-melis Galactomyces geotrichum* [11]. In this study *Pseudomonas fluorescens* and *Bacillus spp.* were chosen to treat the whey because they are encountered in the laboratories of bacteriology, widespread in the environment, lives primarily in water and moist soils and they are not pathogenic. Both bacteria live without difficulty at ambient temperature of 23°C to 35°C and pH near neutral (pH of the whey), they are easy to grow under aerobic conditions at room temperature, to oxidize glucose and to degrade nitrate, starch and casein. [1]

2. Materials and Methods

The parameters used in this project to determine the efficiency of the sweet whey treatment and the degradation of organic matter were chosen according to the most wastewater treatment studies realized in the literature.

2.1. Sampling

Sweet whey samples were collected from the dairy industry "MOAWAD Foundation" located in North Lebanon after the draining step of the cheese. Part of the samples was conserved in the refrigerator, the other one was congealed.

2.2. Whey characterization

The physicochemical analysis of sweet whey was realized according to the methods described in AFNOR and QUEBEC standards including the following parameters: temperature, pH, conductivity, hardness, total suspended solid (TSS), Chemical Oxygen Demand (COD), Total nitrogen (N-NTK), nitrate, nitrite and total phosphorus (P-PT).

2.3. Physicochemical treatment of sweet whey (coagulation, flocculation)

Different coagulation flocculation trials were realized to the sweet whey on ambient temperature (24± 2 °C) using a Jartest according to the following principle: in a series of beakers containing sweet whey, coagulating agents (600mg/L d'Al_2SO_4) [5] are introduced in the sweet whey and stirred for a short period at low speed (200 rpm for 10 min) ensuring a good spread of coagulating agents and a chemical destabilization of colloids. Then the flocculent was added (2.5 mg/L d'alginate de sodium), the whey was stirred gently (40 rpm for 20 min) to facilitate the contact of the particles and avoid breaking the flocks formed. Finally, these flocks were settled in a period of 30 min.

The effectiveness of this treatment was evaluated analytically by monitoring the turbidity (measured in FTU: formazin turbidity unit), TSS, COD, Total N, Total P and nitrate.

2.4. Biological treatment of sweet whey

This treatment is realized by inoculating a sample of whey (500 ml) with *Bacillus spp.*, another sample with *Pseudomonas fluorescens* and a third sample with a mixture of these two bacteria. The mixture is carried out in order to study the effectiveness of these bacteria used together in the same medium to treat whey and to degrade organic matter. These samples were placed in the shaker for 20 days at 100 rpm and at room temperature. Another sample of sweet whey (500 ml) was not inoculated with bacteria, is also placed with the other samples in the shaker under all conditions. This sample was studied in order to determine the ability of autochthonous bacteria to degrade organic matter. All the samples were oxygenated once per week.

In order to follow the growth and chart the growth curve of *Bacillus spp.* and *Pseudomonas fluorescens* two conical flasks containing each one a liquid nutrient medium (Muller Hinton), one is seeded with *Bacillus spp.*, the other one with *Pseudomonas fluorescens*.

The effectiveness of the biological treatment and its monitoring are studied by measuring the following parameters: Absorbance, COD, TSS, Total nitrogen, Total phosphorus and nitrate.

3. Results and discussions

3.1. Characterization of sweet whey

In order to investigate the ability of the physicochemical and biological process to treat the sweet whey, several characteristics of this whey have been studied. The pH of the sweet whey is 5.99, TSS 2850 mg/L, COD 60000 mg/L, Total nitrogen 440 mg/L, Total phosphorus 1490 mg/L and nitrates 320 mg/L. These results show that sweet whey is characterized by an extremely high organic load, nitrogen, phosphorus and suspended solid.

3.2. Physicochemical treatment

The physicochemical treatment by aluminum sulphate and sodium alginate shows a significant reduction of 45% of turbidity, 72 % of TSS, 47.5% of nitrates and a moderate reduction of COD (23.4%), total phosphorus (20%) and total nitrogen (16.4%).

3.3. Biological treatment

These two growth curves show that *Bacillus spp.* and *Pseudomonas fluorescens* in the whey grow over time and remain alive after 20 days, compared to those of nutrient broth that died after this time. This also indicates that the sweet whey can be considered a favorable environment for the growth of these two bacteria.

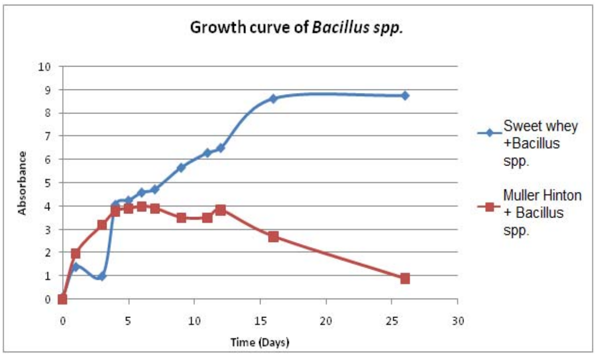

Fig. 3: Growth curve of *Bacillus spp*. in the sweet whey and Muller Hinton

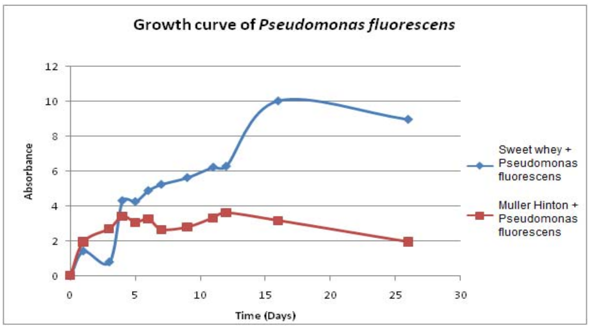

Fig. 4: Growth curve of *Pseudomonas fluorescens* in the sweet whey and Muller Hinton

COD was studied during the biological treatment to monitor the degradation of organic matter and the effectiveness of this treatment.

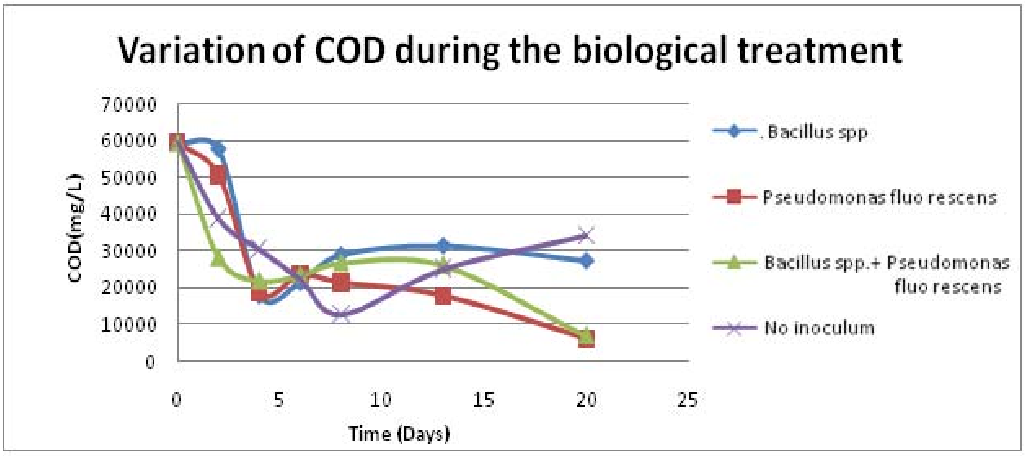

Fig. 5: Variation of COD during the biological treatment by *Bacillus spp.*, *Pseudomonas fluorescens* and their mixture

This chart shows that the COD is reduced especially in the treatment with *Pseudomonas fluoresens* up to 90%, and the treatment with the mixture reduced 88% of COD for 20 days. But under the same conditions, *Bacillus spp.* has reduced the COD with just 54% only. This indicates that *Pseudomonas fluorescens* was most effective in the treatment of sweet whey under these conditions.

After the biological treatment, *Bacillus spp.* and *Pseudomonas fluorescens* reduced 92% of the total nitrogen and around 75 % of nitrates.

4. Conclusion

Wastewater from dairies and cheese industries contain mainly organic and biodegradable materials that can disrupt aquatic and terrestrial ecosystems. Hence the importance of carrying out a whey treatment as a starting point in order to optimize a simple and economic method to treat the whole dairy effluent.

The physicochemical treatment realized in this study was effective reducing 45 % of the turbidity, 72 % of TSS, 23.4 % of COD and 20 % of total phosphorus, while the biological treatment reduced 90 % of the COD, 92 % of the total nitrogen especially when the whey is treated with *Pseudomonas fluorescens*. But we cannot ignore the fact that Bacillus reduced 54 % of the COD.

This sweet whey treatment studied shows a simple, easy, realizable, economic and low cost method applicable on industrial scale.

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