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EFFECTIVENESS OF BACILLUS SUBTILIS STRAIN IN THE TREATMENT OF DAIRY INDUSTRY WASTEWATER

Annotation: *The biological treatment of the B.subtilis (Bacillus Subtilis) ATCC: 6633, bacterial strain was applied for treating Homs Dairy Company at a temperature*

25°c, at pH= 6.5 for the medium and concentration of the strain 10^6 cfu/ml with the presence of an air pump and continuous stirring to ensure homogeneity. The value of COD (Chemical Oxygen Demand) was measured throughout the treatment period which was 14-days. The study was carried out in two ways. The first one, without a preliminary treatment for the wastes. The second, using autoclave sterilization as a preliminary step. As a rustle of the sterilization, the COD value decreased from 10^4 mg / l to 6400mg /l. The percentage of the COD removal for the first treatment was 68% compared with 90% after sterilization under the same conditions. The bacterial strain B.Subtilis was effective in reducing the large quantity of organic materials of Dairy waste of Homs Company.

Keywords: *B.Subtilis, Biological treatment, Chemical Oxygen Demand COD).*

ЭФФЕКТИВНОСТЬ ШТАММА BACILLUS SUBTILIS ПРИ ОЧИСТКЕ СТОЧНЫХ ВОД МОЛОЧНОЙ ПРОМЫШЛЕННОСТИ

Аннотация: *Биологическая обработка B.subtilis (Bacillus Subtilis) ATCC: 6633, бактериальный штамм был применен для лечения Молочная компания Хомса при температуре 25ос, при pH 6,5 для среднего и концентрации штамма 106 цфу/мл с наличием воздушного насоса и непрерывного перемешивания для обеспечения однородности. Значение ХПК (Химический спрос на кислород) измерялось на протяжении всего периода лечения, который составлял 14 дней. Исследование проводилось двумя способами. Первый, без предварительной обработки отходов. Во-вторых, использование автоклав стерилизации в качестве предварительного шага. В качестве шелест стерилизации значение ХПК снизилось со 104 мг/л до 6400 мг/л. Процент удаления ХПК для первого лечения составил 68% по сравнению с 90% после стерилизации в тех же условиях. Бактериальный штамм B.Subtilis был эффективен в сокращении большого количества органических материалов молочных отходов компании Хомс.*

Ключевые слова: *B.Subtilis, Биологическая обработка, Химический спрос кислорода COD).*

1.Introduction:

Nowadays, the industry has to consider further the environmental standards by controlling the composition of the industrial waste and studying its impact on the flora and fauna of the environment. The food treatment industry has faced a problem evolved over recent years becoming more and more crucial. It is the pollution created by the waste and releases of this industry [1]. In Syria, many food industries, a large consumer of water, throw their highly polluting effluents directly in unfit to any self-treatment including drought deemed wadis.

The dairy industry is one of the most polluting industries, not only in terms of the volume of effluent generated, but also in terms of its characteristics as well. It generates about 0.2-10 liters of effluent per liter of processed milk [2] with an average generation of about 2.5 liters of wastewater per liter of processed milk [3]. In the dairy industry, some amount of wastewater produced during starting, equilibrating, stopping and rinsing of the treatment units. Dairy wastewater contains milk solids, detergents, sanitizers, milk wests and cleaning water. It is characterized by high concentrations of nutrirnts and organic and inorganic contents [4]. The quality and quantity of the product content in the dairy wastewater is changing according to the technological used [5]. The total COD of dairy waste water is mainly influenced by the milk, cream, or whey [6]. The value of pH range from4.7to 11[7]. whereas the concentration of suspended solid (SS) varies in the range from0.024 to 4.5 g/l. The nitrogen in dairy wastewater produced mainly from milk proteins. Phosphorus is found mainly in inorganic forms such as orthophosphate (PO_3^{-3}) and polyphosphate($P_2O_7^{-4}$).as well as, in organic forms [8].

Syria is an important contributor to the dairy industry. However, Homs Dairy Company wastewater is estimated at around 150 m^3/day. This company discharges huge amounts of waste that have environmental consequences. A lot of Studies referred

to use Microorganisms in the treatment of dairy wastewater, some of it used *Bacillus. Spp* [9], *B. Subtilis* immobilized in sawdust [16], and used three types: *B. coagulans, B.Simplex, and T.pepton*[17]. Microorganisms contributed in the reduction of environmental pollution.

Our current study aimed at testing the ability of *Bacillus Subtilis* to degrade the organic materials of Dairy Homs Company wastewater.

2.MATERIALS AND METHODS

Materials:

Dairy wastewater used in this study was obtained from Homs Dairy Company, Homs, Syria. The effluent samples were collected from the production hall and Kashkawan industry department of Homs Dairy Company, then transported to the laboratory. The temperature and the pH of the samples were measured at the site. The microorganism used in the experimental tests were two bacterial strains (*B.Subtilis* and *E.coli*) and two strains of fungi (*Aspergillus Brasiliensis* and *Candida Albicans*).

The Physicochemical characteristic of the samples before and after a biological treatment have to be found with reference to the following parameters. The Chemical Oxygen Demand (COD) was determined in the laboratory using a spectrophotometer of the HANA instrument model HI83099. The Dissolved Oxygen (DO) was determined by using the Winkler's Method [11]. The value of COD (Chemical Oxygen Demand) was measured throughout the treatment period which was14-days, the study was carried out in two ways:

1- without a preliminary treatment for the wastes.

2- Using autoclave sterilization as a preliminary step (a decrease in the COD value was observed after the sterilization), with the presence of an air pump and continuous stirring to ensure homogeneity.

Sample specifications: initial COD = 10000 mg/l, pH = 6.5 and DO = 2.88 mg/l

Operational conditions: T = 25°C and C = 10^6 cfu/ml.

Growth Condition of Bacteria

Bacillus.Subtilis ATCC: 6633 was generously obtained from the Faculty of Science, Microbiology section, Al Baath university.

B. Subtilis was maintained on nutrient broth a good growth of bacteria was observed after 24 h. Tryptone soya broth was used as specific cultural media.

After preparation and sterilization of this medium, 1 ml of enrichment culture was added to it and shacked for 24-48 hr at 30 °C. After complete growth of the microorganisms, they were transferred to the sterilized solid cultures tryptone soy agar (TSA) and plate count agar, and incubated at 30 °C.Serial Dilution was made and the efficient concentration was at 10^8 cell/ml in bacterial suspension. whereas, the concentration used in treatment was 10^6 cfu/ml.

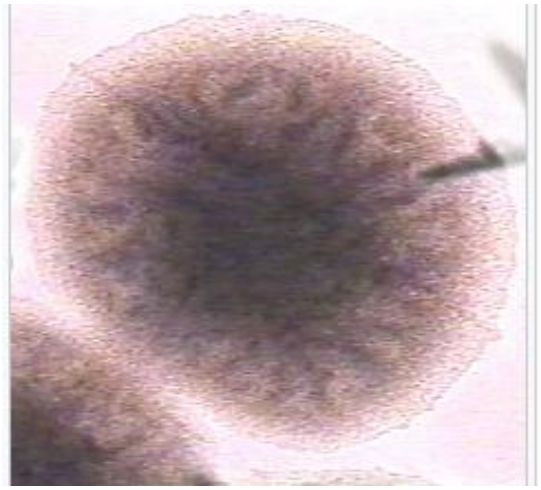


Fig.1: B.Subtilis colony in TSA. Fig.2: The experimental unit used in biological treatment

3.Results and Discussion:

Biological treatment :The experimental test showed that the *B.Subtilis* and *C.Albicans* were efficient in the reduction of COD (77.1% and 86.3% , respectively), and due pathological and ecological effects of *C.Albicans* used *B.Subtilis* .so , it selected to treatment. Table 1 and Fig. 3 shows the reduction of the COD during 14 days. The ability to reduce COD by *B.Subtilis* without a primary treatment of the wastewater was 68%. While Table .2 and Fig.4 show the ability to reduce COD increased to 90 % when Using autoclave sterilization as a preliminary step. as well as,

after sterilization the value of COD decreased from 10000 mg / l to 6400mg /l, which can be explained by a part of organic materials was oxidized during sterilization.

PH :The obtained results show that pH values moved towards the neutrality in biological treatment. It was clear that both without a primary stage and with a primary stage (using autoclave) have the same effect on the changes of pH value table. 1 and table. 2.

Table .1: shows the variables studied during the treatment (Non-Sterile Dairy Effluent)

day	pH	DO	COD
1	6.50	2.73	9100
2	6.65	2.44	6400
3	6.72	2.00	5500
4	7.20	1.70	4600
5	7.53	1.63	3200
7	7..61	1.52	3200
8	7.55	1.50	3200
9	7.51	1.51	3155
10	7.56	1.43	3150
11	7.53	1.51	3150
14	7.48	1.49	3100

Table .2: shows the variables studied during the treatment (Sterile Dairy Effluents)

day	pH	DO	COD
1	6.75	2.61	5200
2	7.13	2.29	2280
3	7.38	2.09	1250
5	7.61	1.80	640
6	7.70	1.59	640
7	7.81	1.37	320
8	7.88	1.30	960
9	7.90	1.20	640
10	8.06	1.20	640
11	8.10	1.50	640
14	8.12	1.59	640

Studies of the biodegradation of dairy wastewater using microbial isolates obtained from activated sludge showed the same changes in pH values [15]. The change in pH values may be attributed to the ability of Bactria to accumulate organic acids after the biodegradation process [13].The variations in pH may affect the degradation of organic materials by Bactria [10]. So, the variations in pH were monitored during the study. There was a slight increase in the pH till the fifth day. The increase could be due to the degradation of fatty acids and organic acids having COO^{-} and OH groups. Moreover, the microbial activity involves the degradation of protein and releases $NH4^{+}$ ions which causes the increase of pH [12].

COD and DO :According to the results in Table .1 and Table. 2, The observed removal (without a primary treatment and Using autoclave sterilization as a preliminary step) was (68% and 90.5%, respectively).

The reduction in COD values may be due to the presence of high concentrations of nutrients and dissolved organic materials which can be easily used by Bactria for growth. The obtained results were in accordance with the results obtained by Chatterjee & Pugaht [14].

The highest reduction of COD was obtained in the fifth day of treatment, the COD remains slightly high after treatment; this verifies that a high amount of organic materials as a substrate is still present in the media. After 5 days, biomass goes into the exponential phase with a very slow growth.

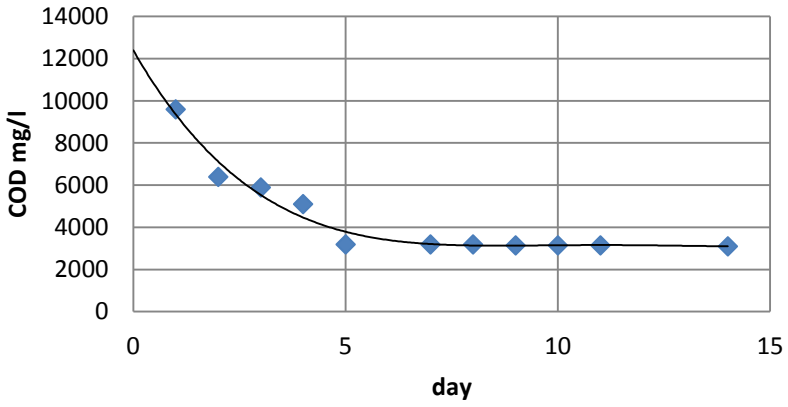


Fig.3 shows the reduction of COD during the biological treatment

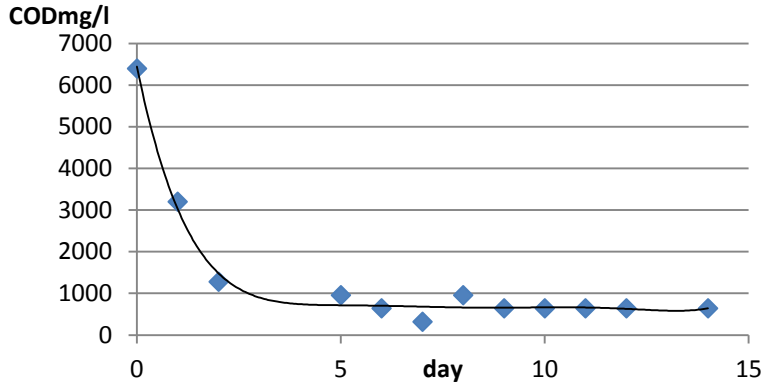


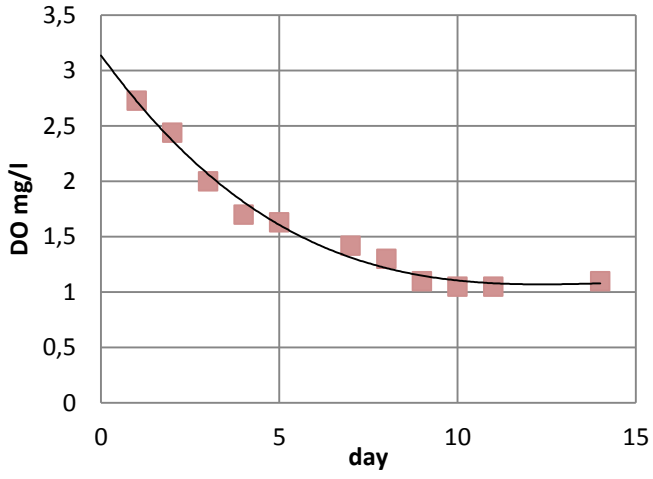
Fig.4 shows the reduction of COD during biological treatment (Sterile Dairy Effluents)

The reduction in COD can be explained by the use of organic matter present in the media by Bactria to meet their energy needs required for cellular biosynthesis reactions in the presence of oxygen [9].Fig.3 the starting value of COD was 10,000 mgO_2 / l, while it reached 3200 mg / l after five days of treatment.

The reduction of DO during biological treatment, a decrease in the DO values similar to the change in COD values (Fig.3 and Fig.4).

Initial DO of the sample was 2.88 mg/l, there was a decrease in the DO till the fifth day. The reduction of DO can be explained by the Bacteria needs for oxygen for the simultaneous utilization of organic materials necessary for growth and other activities [9].

Fig.5: change oxygen dissolved with time (day)



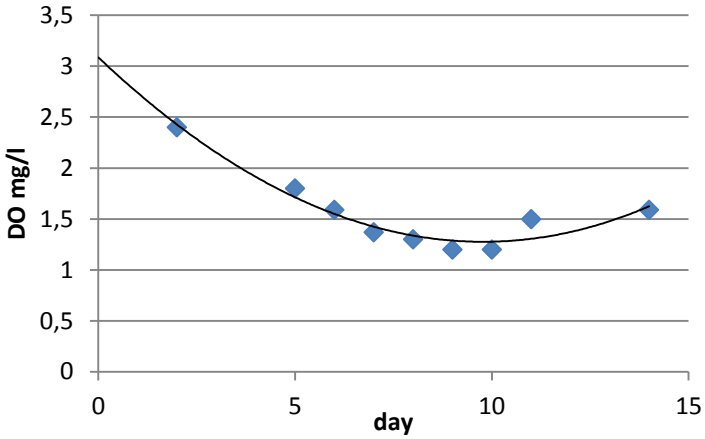


Fig.6: change oxygen dissolved with time (Sterile Dairy Effluent)

From Figure (6) it appears that the decrease in DO values is similar to the decrease in the COD values over time..

Conclusion

From the results which are obtained from this study, it can be concluded that biological treatment of dairy effluent is effective especially in the case of using autoclave sterilization as a preliminary step.

The removed percentage of pollutants present in dairy effluent was increased. when the wastes are subject to preliminary treatment using autoclave as it reached COD = 640 mg / l. While it did not reach the standard when used the wastes directly from the production hall, the value of COD = 3200 mg / l.]

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