

## The use of Anaerobic Digestion Process in the Treatment of Dairy Wastewater by Microorganisms Derived from Sewage Wasted Sludge

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### Abstract

The aim of this work is to determine the potential use of sewage wasted sludge (SWS) as a source for heterogeneous microorganisms to be used in dairy wastewater treatment by anaerobic suspended digestion process. A lab scale stainless steel reactor was designed with dimensions of 600 mm height and 350 mm inner diameter. Different operating conditions of the treatment process were studied such as the organic loading rate (OLR), temperature and hydraulic retention time (HRT). For 14 days operation period, the results of the anaerobic digestion were expressed in terms of COD, BOD, TDS and TSS reduction. The effect of temperature was investigated by operating the reactor at temperature range of 20-40°C and the optimum reduction has been observed at 35 °C. The maximum removal efficiency was obtained at an OLR of 0.5 kg/m<sup>3</sup>.day and 72 hr HRT, at which COD, BOD, TDS and TSS were removed by 80%, 88%, 90% and 93% respectively.

Keywords: Sewage Wasted sludge (SWS), Heterogeneous microorganisms, Anaerobic digestion Dairy wastewater.

### الخلاصة

أهداف من هذه الدراسة هو تقييم أمكانية استخدام مخلفات الحماة للمياه المتخلفة كمصدر لمختلف الأحياء المجهرية لاستخدامها في معالجة مخلفات مياه الألبان بواسطة عملية الهضم اللاهوائي. تم تصميم مفاعل مختبري من الحديد المغلوق ذو أبعاد 600 ملم ارتفاع و 350 ملم قطر داخلي. تمت عملية المعالجة تحت ظروف تشغيلية مختلفة وهي معدل الأحمال العضوية (OLR)، درجة الحرارة وفترة المكوث الهيدروليكي (HRT). بعد فترة تشغيل 14 يوم، فإن نتائج الهضم اللاهوائي تم التعبير عنها بدلالة المتطلب الكيميائي للأوكسجين (COD)، المتطلب الحيوي للأوكسجين (BOD)، المواد الصلبة الذائبة الكلية (TDS) و المواد الصلبة العالقة الكلية (TSS). تأثير درجة الحرارة تم اختبارها عن طريق تشغيل المفاعل تحت مدى تراوح بين 20-40 م° وأفضل أزاله لكل المحددات المدروسة كانت عند درجة حرارة 35 درجة سيليزية. أكبر أزاله تم الحصول عليها عند معدل حمل عضوي (OLR) مقداره 0.5 kg/m<sup>3</sup>.day وفترة مكوث هيدروليكي مقدارها 72h حيث تم عندها أزاله المتطلب الكيميائي للأوكسجين (COD)، المتطلب الحيوي للأوكسجين (BOD)، المواد الصلبة الذائبة الكلية (TDS) والمواد الصلبة العالقة الكلية (TSS) بنسبة 80 %، 88%، 90% و 93% على التوالي.

## 1 Introduction

The dairy industry, like most other agro-industries, generates strong wastewaters characterized by high biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations reflecting their high organic content<sup>[1]</sup>. Dairy waste effluents are concentrated in nature, and the main contributors of organic load to these effluents are carbohydrates, proteins and fats originating from the milk<sup>[2]</sup>. Dairy wastewater is invariably high in nutrients (i.e. nitrogen, phosphorus and potassium) and organic material (e.g. oils and fat, dissolved lactic acid, etc.) and consequently has a high biological oxygen demand (BOD). Furthermore, dairy-processing effluent also has high concentrations of dissolved salts (total dissolved solids, TDS). The use of acid and alkaline cleaners and sanitizers in the dairy industry additionally influences wastewater characteristics and typically results in a highly variable pH<sup>[3]</sup>. According to EPA Feedlots Point Source Category Study (EPA, 1999), dairy farm wastewater has average chemical oxygen demand (COD) concentrations of 4997 mg/L and biochemical oxygen demand (BOD<sub>5</sub>) of 1003 mg/L. The COD concentration varies in the range of 2000– 7000 mg/L depending on wastewater management, climate, operation conditions, and types of flushing. The high COD concentration is due to milk waste (produced by washing milking equipment), detergent, manure, and waste feeds combined in the washing or flushing of holding pens and exit alleys<sup>[4]</sup>. According to Demirel et al., (2005) 0.7–1.7 m<sup>3</sup>/ton of milk wastewater generates a BOD<sub>5</sub> concentration of 500–1500 mg/L<sup>[5]</sup>.

Environmental problems associated with animal manure and wastewater are the quality degradation parameters affecting surface water (e.g., lakes, streams, rivers, and reservoirs) and groundwater, adverse effects on estuarine water quality and resources in coastal areas, and effects on soil and air quality<sup>[6]</sup>. A large number of treatment techniques practiced on dairy farm wastewater and manure management were transferred directly from the municipal wastewater treatment field that may not be appropriate for the application in dairy wastewater management systems. The application of conventional methods: aerated lagoons, activated sludge processes, trickling, and rotating biological contactors on dairy wastewater proves to be difficult and complex processes, which also consumes a great deal of energy at low efficiency rates. Therefore, a treatment/reuse system for dairy wastewater is needed to be developed as a more appropriate one for land-limited conditions, i.e., island applications<sup>[6]</sup>.

Anaerobic digestion (AD) is among the oldest processes used for the stabilization of solids and biosolids<sup>[7]</sup>. Both European community and U.S. consider the anaerobic treatment as the most promising approach for future in sustainable development<sup>[8]</sup>. Based on the principle of separating different flows of domestic, anaerobic methods for dairy wastewater treatment have currently become the focus of a great deal of researches attention<sup>[9, 10]</sup>. Several factors make anaerobic treatment a good choice for the treatment of dairy wastewater, high

organic concentrations, the low energy requirements for anaerobic processes, reduce sludge production, and the generation of methane gas<sup>[11]</sup>. To this effect, anaerobic digestion offers a unique treatment option to the dairy industry. Not only does anaerobic digestion reduce the COD of the effluent, but little microbial biomass is produced. The biggest advantage is energy recovery in the form of methane gas and up to 95% of the organic matter can be converted into biogas<sup>[10]</sup>, also no oxygen is required in anaerobic digestion so COD is converted to methane<sup>[2]</sup>. The anaerobic digestion of food waste can reduce its volume, generate fuel biogas containing methane and produce solid organic residue that can be used as soil conditioner or fertilizer<sup>[4]</sup>. There are many kinds of anaerobic processes, one of them is the anaerobic contact process, which depends on a suspended growth technique with recycling (not completely mixed). The biomass is separated and returned to the reactor so the SRT is longer than the HRT<sup>[5]</sup>. *In the present study*, anaerobic sludge was used as a source for anaerobic microorganisms. This sludge is the biomass waste generated from the regular biological activities of municipal wastewater treatment plants. The volume of this sludge is being increased with the increasing of the treated municipal wastewater. This waste material seems to be a promising way of turning it into a useful resource of heterogeneous microorganisms, like bacteria, fungi, yeast and protozoa<sup>[12]</sup>.

## **2. Materials and methods**

### **2.1 Dairy wastewater**

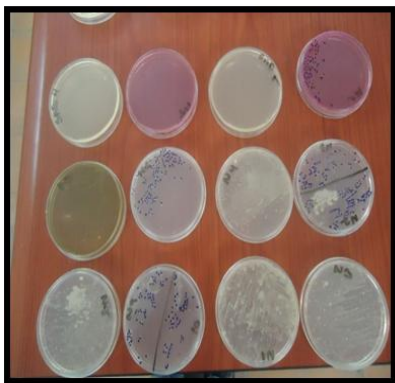
The dairy wastewater used in this study was collected from a factory affiliated to the College of Agriculture at Baghdad University. The factory suffers a default in the treatment of its wastes from the production of cheese, cream, ice-cream, yoghurt and pasteurized milk. The dairy wastewater represents the accumulation of whey, factory spills and washwater from the cleaning cycles. After samples collection, the wastewater was transferred immediately to the laboratory and stored at 4 °C until it was tested. The average characteristics of the dairy effluent were measured at University of Al-Mustansiriya/College of Engineering/Sanitary Engineering laboratory and listed in table 1.

**Table 1 Average Dairy Effluent Characteristics**

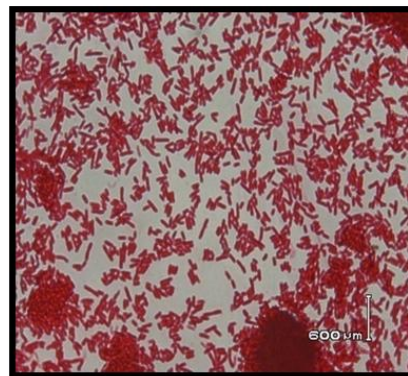
Parameter	pH	COD mg/L	BOD <sub>5</sub> mg/L	TSS mg/L	TDS mg/L	VSS mg/L	N mg/L	P mg/L
Value	5	1500	900	3750	850	580	84	12

## 2.2 Microorganism and growth conditions

The anaerobic sludge used in the present study was collected from the sewage sludge collection system of Al-Rustamiyah sewage treatment plant in Baghdad city. The sludge was taken from the drying beds, the volume of sludge produced is about 7500 m<sup>3</sup> every 20 days. The sludge is collected from about 20 cm depth of drying bed surface where anaerobic conditions are predominate. The observation of methane gas bubbles during the collection of the sludge proved this fact. To identify species of microorganisms present in anaerobic sludge, the sludge was serially diluted with distilled water. The microorganisms found in the sludge were heterogeneous as listed in table 2 and consists mainly of anaerobic bacteria, yeast, fungi and protozoa according to the ASTM biochemical tests [13]. Figures 1 and 2 show the appearance of microorganisms in the tested sludge. Concentration of microorganisms expressed by organic volatile solid (V.S) in the anaerobic sludge was measured to be  $0.135 \times 10^6$  mg/L. These tests were conducted at the laboratories of the Ministry of Environment, Environmental engineering department/Al-Mustansiriya University and College of Science/Baghdad University.



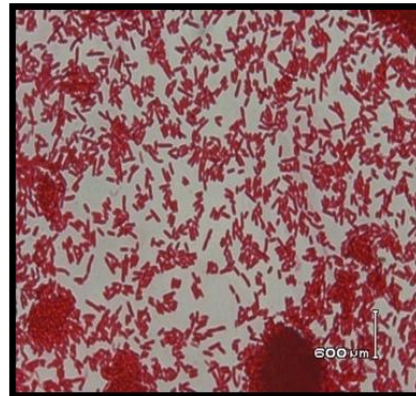
**Fig. 1** Petri dish of different nutrients agar where microorganisms cultivate



**Fig. 2** Microorganism appearance in light microscope.



**Fig. 1** Petri dish of different nutrients agar where microorganisms cultivate



**Fig. 2** Microorganism appearance in light microscope.

**Table 2 Identified species of microorganisms in the sludge**

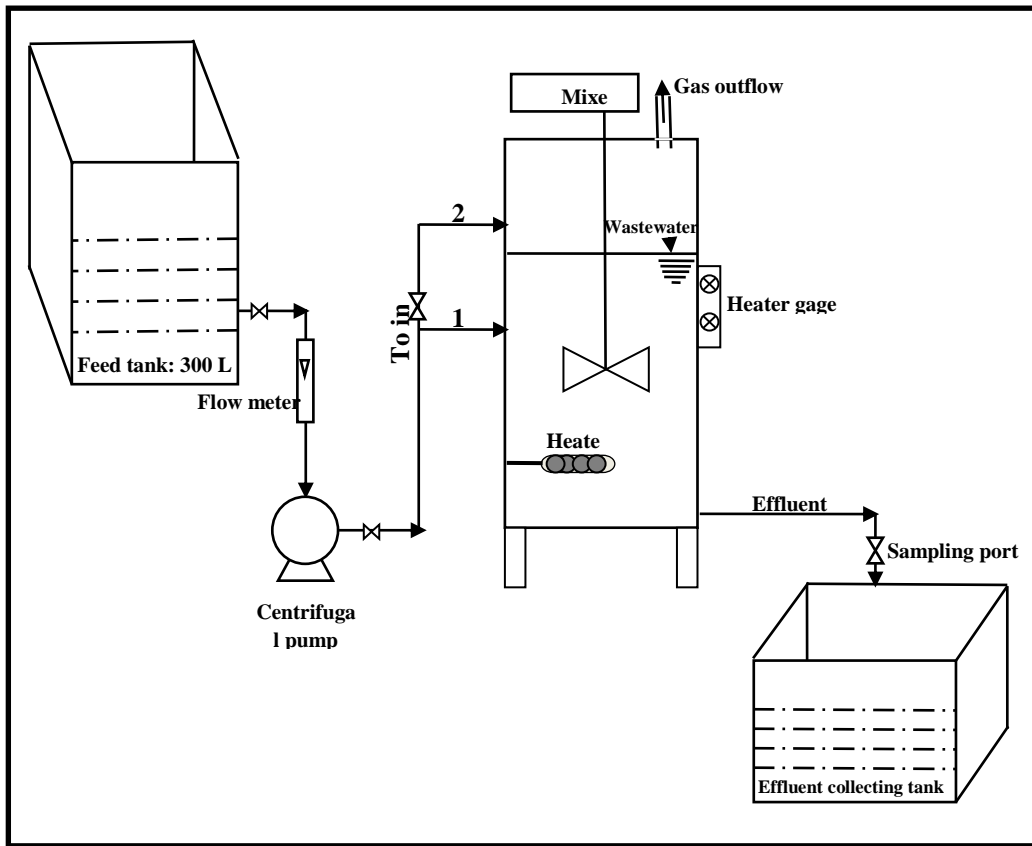
<b>Bacteria</b>	<b>(CFU/ml)</b>
<i>Aeromonas sp.</i>	$2.22 \times 10^5$
<i>E-coli</i>	$4.3 \times 10^5$
<i>Pseudomonas aerginrsa</i>	$7.04 \times 10^5$
<i>Klebsiella species</i>	$2.1 \times 10^5$
<i>Clostridium</i>	$3.7 \times 10^5$
<i>Staphylococcus sp.</i>	$2.1 \times 10^5$
<i>Streptococcus sp.</i>	$4.9 \times 10^5$
<i>Salmonella sp.</i>	$1.9 \times 10^5$
<i>Shiglla dysentery</i>	$4.1 \times 10^5$
<b>Fungi</b>	<b>(CFU/ml)</b>
<i>Penicillium sp.</i>	$5 \times 10^4$
<i>Yeast</i>	(CFU/ml)
<i>Candida albicans</i>	$2.1 \times 10^4$
<b>Protozoa</b>	<b>(CFU/ml)</b>
<i>Entamoeba species</i>	$1.5 \times 10^3$
<i>Giardia lambihia</i>	$6.5 \times 10^5$

### 2.3 Experimental Procedures

A stainless steel cylindrical reactor was designed and constructed with dimensions of 600 mm in height and 350mm inner diameter, supplied with a mixer and heater. It was connected to a storage tank and effluent tank (for sludge recycling). A schematic representation of the experimental equipment is shown in Fig.3. The laboratory unit is depicted in Fig.4. The reactor was partially filled with waste up to two third of its volume. The volume of the liquid microbial culture inoculated into the reactor was 100 ml and seeded with a nutrient medium of 2000 mg/L NaCH<sub>3</sub>COO as the sole carbon source, 500 mg/L



$\text{NH}_4\text{NO}_3$ , 500 mg/L  $\text{KH}_2\text{PO}_4$ , 200 mg/L  $\text{CaCl}_2$  and 200 mg/L  $\text{MgSO}_4$  for microorganisms growth<sup>[14]</sup>.



**Fig. 3 Schematic diagram of the anaerobic reactor**



**Fig. 4 The basic connection of the anaerobic contact system**

Anaerobic digestion has a small pH tolerance range of 6.7 to 7.4 with optimum operation at pH 7. So, pH was adjusted with the range of 7 for all experiments by adding 0.1N HNO<sub>3</sub> and 0.1N NaOH for acidic and basic pH respectively <sup>[5]</sup> and the reactor was operated for two weeks.

### 3. Mathematical description

Most anaerobic systems are designed to retain the waste for a fixed number of hours. The number of hours that the materials stays in the tank is called the hydraulic retention time (HRT, h). The hydraulic retention time is important since it establishes the quantity of time available for bacterial growth and subsequent conversion of the organic material to gas <sup>[5]</sup>:

$$HRT = \frac{V}{Q} \tag{1}$$

where V is the volume of treated wastewater, m<sup>3</sup> and Q is the flow rate, m<sup>3</sup>/h.

The solids retention time (SRT, days) is the most important factor controlling the digester stability. Although the calculation of the solids retention time is often improperly stated, it is the quantity of solids maintained in the digester divided by the quantity of solids wasted each day:

$$SRT = \left( \frac{\mu_m S_e}{K_s + S_e} - K_d \right)^{-1} \tag{2}$$

where  $\mu_m$  (g/g.d) is the maximum specific growth rate of the microorganisms that is related to maximum specific substrate utilization rate,  $S_e$  is effluent soluble COD (mg/L),  $K_s$  is the half velocity constant (mg/L) and  $K_d$  is the decay coefficient (g/g.day). For anaerobic digestion process, the optimum operating values of  $\mu_m$ ,  $K_s$  and  $K_d$  are 0.35 g/g.d, 160 mg/L and 0.02 g/g.day respectively <sup>[7]</sup>.

At a low SRT sufficient time is not available for the bacteria to grow and replace the bacteria lost in the effluent. If the rate of bacterial loss exceeds the rate of bacteria growth, "wash-out" occurs. The SRT at which "wash-out" begins to occur is the "critical SRT". The goal of process engineers over the past twenty years has been to develop anaerobic processes that retain biomass in a variety of forms such that the SRT can be increased while the HRT is decreased. The goal has been to retain, rather than waste the biocatalyst (bacterial consortia) responsible for the anaerobic process. As a result of this effort, digester volume decreased. A measure of the success of biomass retention is the SRT/HRT ratio. In conventional digesters, the ratio is 1.0. Effective retention systems will have SRT/HRT ratios exceeding 3.0. At an SRT/HRT ratio of 3.0 the digester will be 1/3 the size of a conventional digester <sup>[5]</sup>.

Neither the hydraulic retention time (HRT), nor the solids retention time (SRT) play the full effects of the impact that the influent waste concentration has on the anaerobic digester. A more appropriate measure of the waste on the digester's size and performance is the organic loading (OLR, kg/m<sup>3</sup>.d) <sup>[5]</sup>:

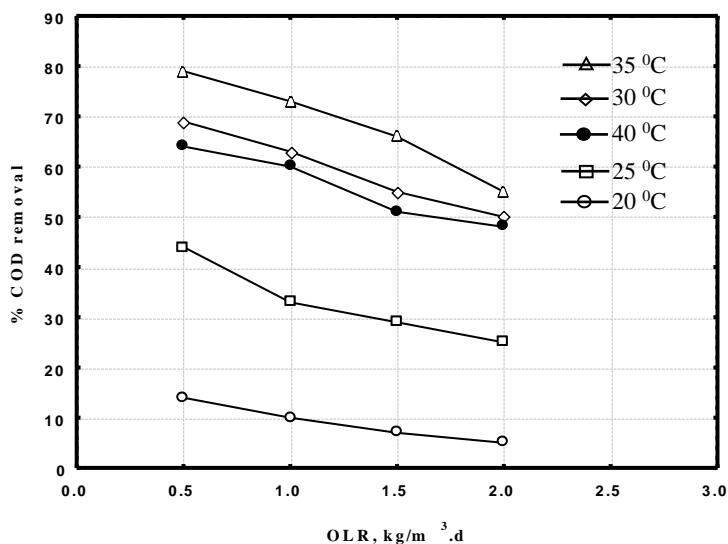
$$OLR = \frac{Q \times S}{V} \tag{3}$$

where S is the influent COD, mg/L.

## 4. Results and Discussion

### 4.1 Effects of Temperature

The dairy wastewater treatment experiments are carried out in the temperature range of 20-40 °C in the thermostatic reactor. According to temperature, microorganisms are classified into three classes. Psychrophilic (0-20 °C), Mesophilic (10-40 °C), and thermophilic (40-75°C)<sup>[15, 16]</sup>. The removal efficiency for varied temperatures at varying organic loading rate and hydraulic retention time of 72 h are shown in Figs. 5, 6, 7 and 8. The percentage COD, BOD, TDS, and TSS have been observed to be minimum at 20°C. However, the results show that, the maximum removal efficiency was achieved at temperature 35°C . The temperature 40°C is closer to the lower range of the thermophilic zone, that is why the percentage removal of all parameters have been observed to be much lesser than that of 35°C temperature. This agrees with Dawood et al., (2011) which studied the anaerobic treatment of synthetic milk wastewater at temperature ranges 15-55 °C. They found that, maximum COD reduction has been observed at 35°C<sup>[17]</sup>.



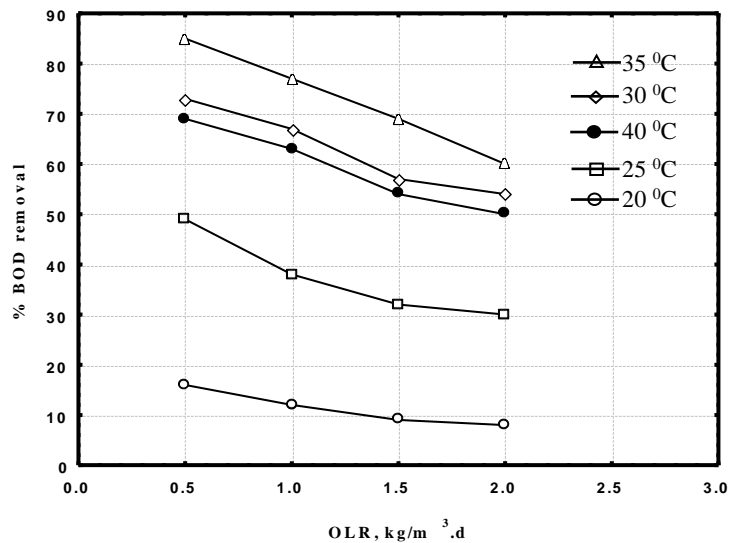
**Fig. 5 COD percentage removal under variable organic loadings at 72 h HRT.**

### 4.2 Effects of organic loading rate (OLR) and hydraulic retention time (HRT)

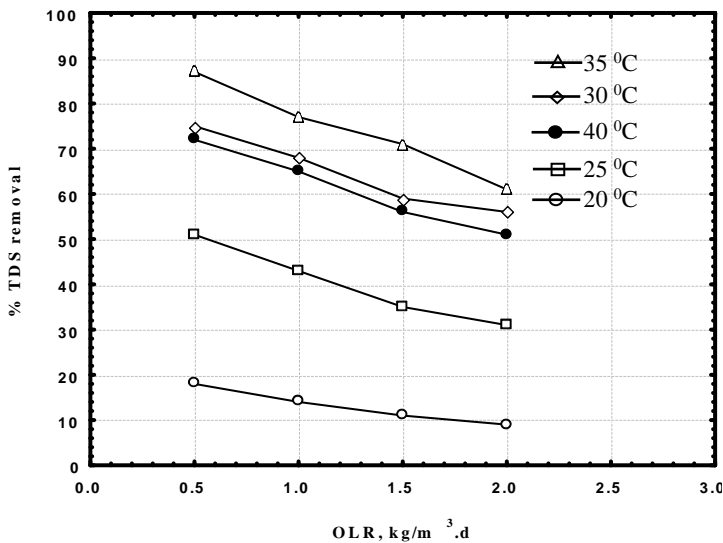
The change in the hydraulic retention time has a significant effect on the removal efficiency curves. Figs. 9, 10, 11 and 12 show the removal efficiency curves at different OLR; 0.5, 1, 1.5, and 2 kg/m<sup>3</sup>.d, and different hydraulic retention times; 18, 24, 48, and 72 h at 35°C. These figures show that, as the HRT increases the removal efficiency of COD, BOD, TDS, and TSS respectively increased. The higher the HRT, the greater the removal efficiency. However; it is clear that increasing HRT increases the residence time of the dairy waste. Similar findings have been obtained by Mohan et al., (2009)<sup>[18]</sup>, Najafpour et al.,



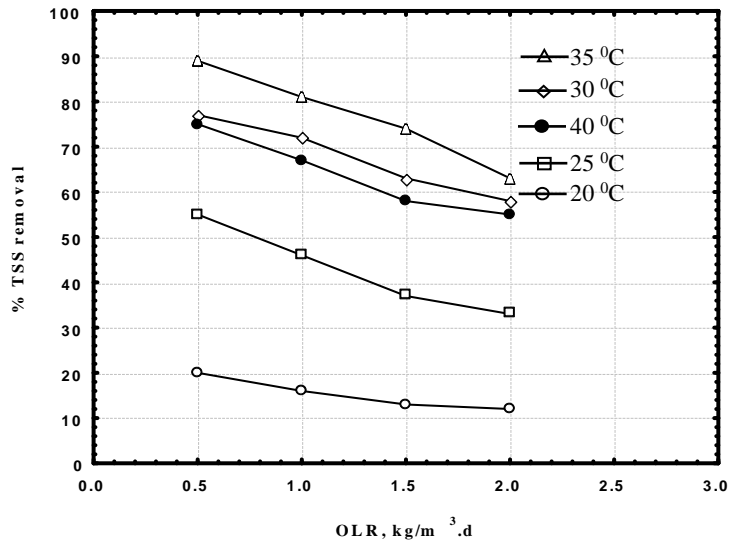
(2006)<sup>[19]</sup> Rajinkanth et al., (2010)<sup>[20]</sup> and Cooper and Aknison<sup>[21]</sup>, Lettinga and Nelson<sup>[22]</sup> and Balasuriya <sup>[23]</sup> they classified that, the optimum reduction of BOD and COD occurred between 8-12 hours for the same ranges of organic loading rate as in this study.



**Fig. 6 BOD percentage removal under variable organic loadings at 72 h HRT**

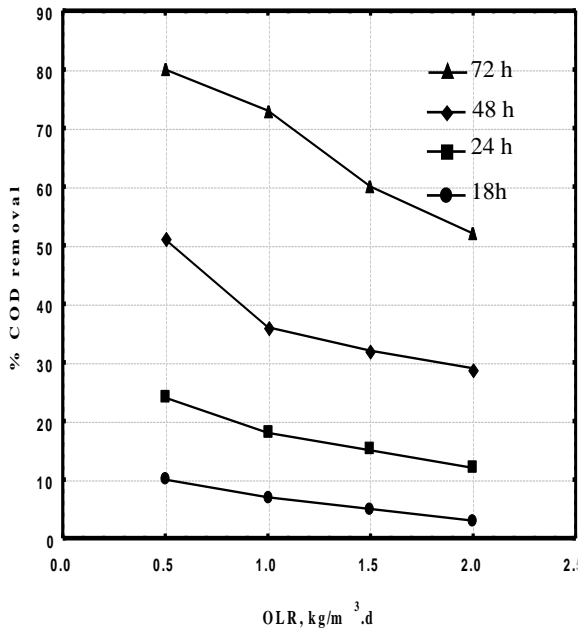


**Fig. 7 TDS percentage removal under variable organic loadings at 72h HRT**

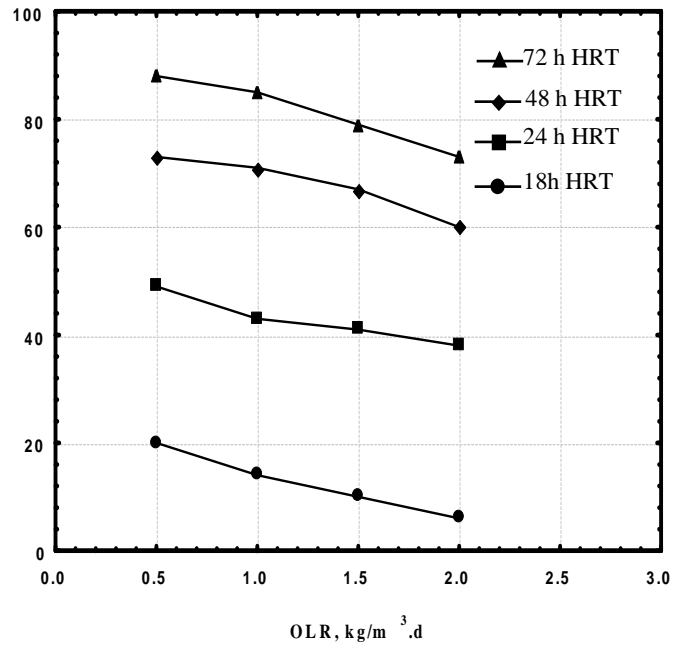


**Fig. 8 TSS percentage removal under variable organic loadings at 72h HRT**

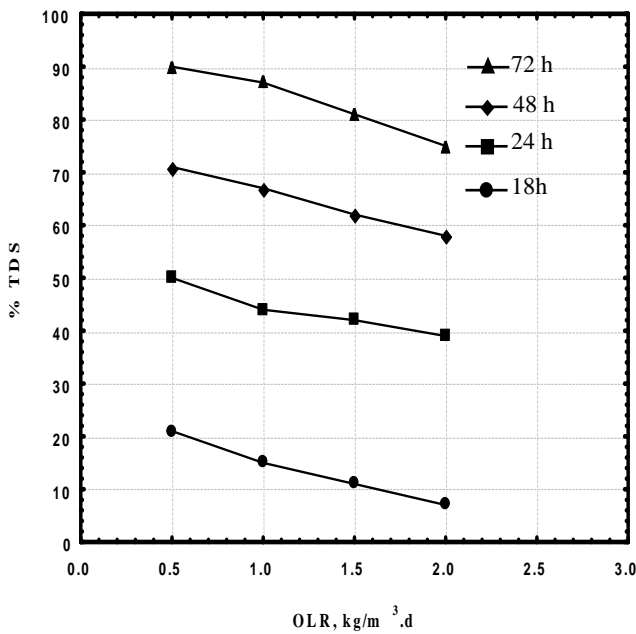
Srinivasan et al., (2009) modified a diphasic digester to treat dairy waste water, they conclude that, diphasic digester can be used for removing COD up to 75% and the rest can be removed in the down stream aerobic systems, more effectively and economically <sup>[24,25]</sup>.



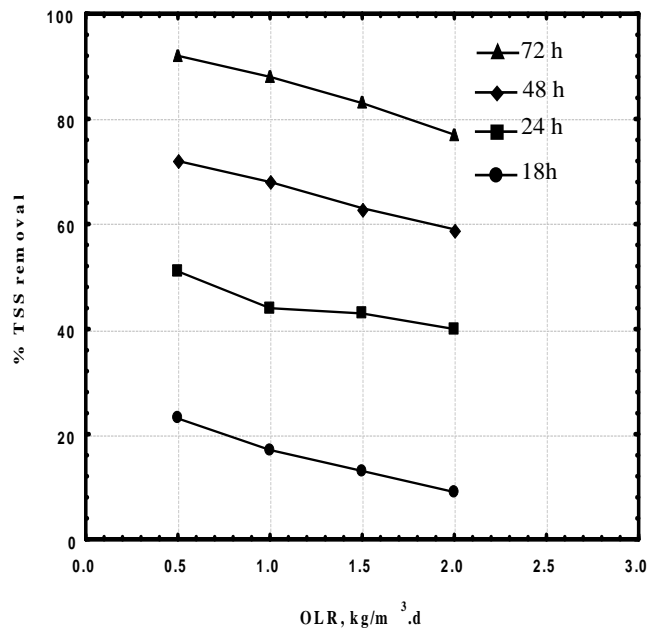
**Fig. 9 COD percentage removal under variable organic loadings at 35 °C**



**Fig. 10 BOD percentage removal under variable organic loadings at 35 °C.**



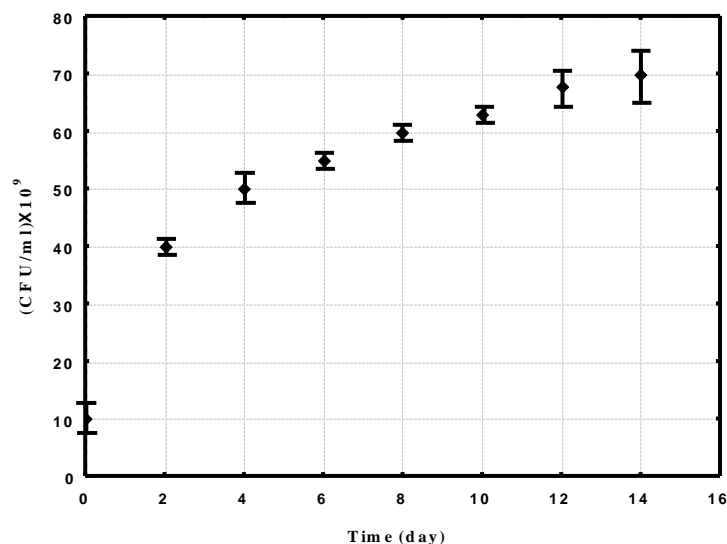
**Fig. 11 TDS percentage removal under variable organic loadings at 35 °C.**



**Fig. 12 TSS percentage removal under variable organic loadings at 35 °C.**

#### 4.3 Microorganisms growth and design parameters of anaerobic reactor

Measurement of optical density as an indication of cell number was achieved during the experiments. Figure 13 shows the results of three replicated experiments. The average standard deviations was found to be 12.3% of the mean biomass. A rapid increase in the cell number occurred in the first 8 days up to  $60 \times 10^9$  CFU/ml combined with rapid decreasing in COD, BOD, TDS, and TSS concentrations. This was followed by a slight increase in cell numbers over the remaining period of the experiment due to the decrease in substrate concentrations. This agrees with the observation for biological treatment of dairy wastewater in an upflow anaerobic sludge-fixed film bioreactor achieved by Najafpour et al., (2008)<sup>[19]</sup>. The solid retention time, SRT is determined from Eq.2 to be 3.50 days, this means that, the SRT/HRT ranged from 1.2-4.7 for a HRT of 18 to 72h respectively. This improves the suitability of the reactor for treating the influent concentrations. In addition, concentration of microorganisms expressed by organic volatile solids (V.S) in the anaerobic sludge used in the present study measured before in the experimental work was  $0.135 \times 10^6$  mg/L. This value is highly more than the concentration in the aerobic sludge which normally doesnot exceed 5000 mg/L<sup>[26, 27]</sup>. The anaerobic microorganisms could resist and live both in anaerobic and aerobic conditions and thus grow in highly concentrations of COD, BOD, TDS and TSS in the treated wastewater which explains the high removal efficiency<sup>[7]</sup>.



**Fig. 13 Average and standard deviation of three replications of cell number.**

## 5. Conclusions

Anaerobic treatment using sludge wastes as a source for heterogeneous microorganisms seems to be a promising way to be used in the reduction of organic pollutants discharged from different industrial sources such as dairy wastewater. Significant COD, BOD, TDS, and TSS reduction at variable organic loadings, variable temperature and hydraulic retention time for dairy wastewater have been found. Experimental results show that, concentrations reduction of COD, BOD, TDS, TSS increases with time period for all the cases considered in this study. As the organic loading rate increases, the percentage reduction for all parameters studied decreased. This phenomenon has been observed in the temperature ranges, 20-40 °C. The maximum removal efficiency has been observed at 35°C, 0.5 kg/m<sup>3</sup>.d OLR and HRT of 72 h. This indicates that mesophilic microorganism work effectively at this range of temperature. Also, as the hydraulic retention time increased from 18-72 h, the detention time for treatment increased and as a result the removal efficiency increased to 80%, 88%, 90% , and 93% for COD, BOD, TDS and TSS respectively. Therefore it's concluded that dairy wastewater and similar industries may be treated efficiently using microorganisms derived from anaerobic wasted sewage sludge.

## Nomenclature

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BOD	Biochemical oxygen demand, mg/L
COD	Chemical oxygen demand, mg/L
CFU	Colony Forming Unit
N	Nitrogen, mg/L
P	Phosphorus, mg/L
TSS	Total suspended solid, mg/L
TDS	Total dissolved solid, mg/L
VSS	Volatile suspended solid, mg/L

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