

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## Experimental Investigation on Biological Treatment of Synthetic Dairy Water.

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### ABSTRACT

The estimated India's milk production in current year 2018 is to increase by around four percent and reaches to 167 million metric tons (MMT). One unit milk product requires five litres of fresh water and generates at an around 3 litres. It is essential to treat the generated effluent water, before discharging into environment. The treatment methods include primary, secondary and tertiary, in which biological treatment process is very much important in the secondary treatment. Biological process involves micro-organisms, it is used to remove non-settleable colloidal solids and stabilise the organic matter. The present study is experimental work done on batch studies and proto type Rotating Biological contactor (RBC) in laboratory for the treatment of synthetic dairy water. The study also involves culturing of bacteria *staphylococcus-aureus*, Sub-culturing of *staphylococcus-aureus* and identification of dry density of organisms. The synthetic dairy water is prepared in the laboratory by using milk powder. The batch studies are used for substrate removal from the prepared synthetic dairy water consisting of 17000 mg/l of COD. A proto type RBC prepared with low cost materials, 4 discs having a total surface area about 0.4 m<sup>2</sup> with 45% submerging in the 6 litres of water. The both experiments batch and RBC are conducted in open atmosphere by adding the above said organism. At the same time a standard sample is also kept in open atmosphere without organism to compare the results. The BOD of the water samples before and after experimental studies are analysed according to IS 3025: part 44 respectively. The batch experiments shows BOD removal efficiency about 77%. Whereas the RBC showed 84% removal efficiency. At the same time RBC got COD removal efficiency about 65%. These results are very much encouraging to work further into continuous process.

**Keywords:** Synthetic dairy water, *staphylococcus-aureus*, Rotating Biological Contactor, BOD, and COD

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**INTRODUCTION**

Dairy industry includes treating of raw milk and many products are prepared like market milk, condensed milk, milk powder, cream, butter, cheese, curd, yogurt and ice cream and many more products are being made [1]. India’s market share of value added dairy products will develop from 20% in 2012-13 to 30% by 2019-20. The robstically development in dairy industry from 1950 to 2011 in India. India’s Milk production in 1950 to 2011 is 17 million tonnes to 121.7 million tonnes [2]. Surface water bodies polluted by small scale industry on that dairy industry is one of most wastewater generated unit and it consisting of highly organic matter. The dairy industry generated waste water is characterised by conducting COD and BOD tests and also BOD test used to find the efficiency of treatment unit [3, 4]. Many of treatment technologies are available in the present world those arechemical treatment, physical, secondary treatment and tertiary treatment on that biological treatment technology is most important in removal of organic matter present in wastewater. Generally biological treatment or secondary treatment widely used biomass such as pseudomonas, actrobacter, arthrobacter, alcaligenes, zooglea, citromonas, flavibacterium and achromobacter for removal of organic matter in wastewater. [5-8].Mostly biological treatment techniques are used high strength (organic matter) or toxic nature of wastewater in continues mode by some constant flow rate and fixed HRT and SRT [9]. The experiment involves synthetic dairy water and the preparation of synthetic dairy wastewater as follows some compositions this are mentioned table number 1and 2. Once the preparation is done it maintain 24 to 90 hours contact time the COD value reaches 17000 mg/l. Based on obtained COD values dilutions are made and experiment was conduct. The COD value is analysed before going start the experiment in every day[10]. The present experiment is a batch type biological treatment unit is used to treat the synthetic dairy wastewater. The present work performed BOD and COD test based on follows IS: 3025 (part 44) – 2003 and 3025 (Part 58) – 2006 [11, 12].The experiment consisting of two stages, they are batch and RBC equipment setup, the equipment setup is prepared in laboratory in a proto type model of 6 litter’s capacity tank. The present work involves Staphylococcus aureus and Escherichia coli, these are the bacteria used to perform biological treatment of batch and RBC. The evaluated results explains Staphylococcus aureus is very effective in break downning of organic matter present in synthetic dairy wastewater at the same time slightly harmful to the humans [13]. The removal efficiencies of Staphylococcus aureus in synthetic dairy wastewater is 84% of BOD and the COD removal efficiency is 74%. The second organism is not good at wastewater treatment as the experiment explains Escherichia coli is slightly increasing the COD and BOD values.

**MATERIALS AND METHODS**

**Preparation of Synthetic Dairy Wastewater**

The prepared synthetic dairy wastewater characteristics show Table 1. The synthetic dairy wastewater is prepared by amul milk powder [3, 8].

**Table 1: Milk powder composition of prepared synthetic dairy wastewater**

S. No	Parameters	Typical Values (mg/l)
1	Total fats	20000
2	Saturated fats	13000
3	Trans fats	1000
4	Total carbohydrates	50000
5	Sugar	18000
6	Protein	20000
7	Ca	820

**Table 2: Additional nutrient solutions present in synthetic dairy wastewater [3, 10]**

S. No	Parameters	Typical values (g/l)
1	NH <sub>4</sub> CL	2.8
2	KH <sub>2</sub> PO <sub>4</sub>	2.0
3	MgSO <sub>4</sub> 7H <sub>2</sub> O	0.1
4	CaCL <sub>2</sub>	0.076

5	NaHCO <sub>3</sub>	4
6	KNO <sub>3</sub>	4

The above mentioned composition are used to prepare synthetic dairy waste water is prepared. The prepared waste water is maintain 96 hours contact time it will reach approximately 17000 ml/l of COD. Make dilutions and prepare required COD values. Every time, before going to start the experiment find out COD value and do the analysis. [10]. The prepared synthetic dairy wastewater make dilutions by the resulting technique as 5000 ml/l, 4000ml/g and 3000 mg/l. The batch studies evaluate each point by the following way. At 5000 concentration to make one standard solution (without microorganisms and open to atmosphere) and sample solution (addition of microorganisms and open to atmosphere). At 4000 concentration to make one standard solution (without microorganisms and open to atmosphere) and sample solution (addition of microorganisms and open to atmosphere). At 3000 concentration to make one standard solution (without microorganisms and open to atmosphere) and sample solution (addition of microorganisms and open to atmosphere). The present batch studies are used Staphylococcus aureus. The Staphylococcus aureus culture of 15 ml solution added to the prepared concentration of synthetic dairy waste sample bottles and placed 5 days. To find out BOD values of first day and fifth day

**Characteristics of microorganisms**

Microorganisms plays a crucial role in the biological treatment of wastewater. The present experiment introduce two microorganisms named Staphylococcus aureus and E-coli in the treatment of synthetic dairy wastewater. The Staphylococcus aureus is harmful to the human beings but it is good in organic matter removal in wastewater treatment. These two microorganisms brought from the AU engineering college. So the present study involves the Staphylococcus aureus and E-coli in the treatment process of synthetic dairy wastewater[14, 15].

**Table 3: Microorganisms Characteristics [13]**

Name	Staphylococcus aureus	Escherichia coli
Gram type	Positive	Negative
Domain	Bacteria	Bacteria
Family	Staphylococcaceae	Enterobacteriaceae
Shape	round shaped	Rod shaped
Size	0.5-1.5 µm diameter	0.5 µm in width by 2 µm in length and 0.25–1.0 µm in diameter
Aerobe or anaerobe	Facultative anaerobe	Facultative anaerobe
Colour	Violet	Dark blue/purple
Diseases cause	Minor skin infections, lung infections or pneumonia, Brain infections, Bone infections, Heart infections	gastroenteritis, urinary tract infections, haemorrhagic colitis, severe abdominal cramps, diarrhoea, haemorrhagic colitis, vomiting, and sometimes fever

**Calculation of Biomass concentrations**

10 ml of sample withdrawn from the subculture sample and centrifuged at 6000 rpm for 20 minutes. Samples from the clear supernatant after centrifugation, biomass concentrations were determined by filtering samples from 0.45 l Millipore filters and determining dry weight after drying in an oven until constant weight[9].

**RBC Equipment setup**

The alternative names of RBC are media, disc, surface and biofilm reactors and provide an alternative to the activated sludge (AS) process. The microbial growth is encourages in the RBC because it existing solid media support through static biofilm. The RBC setup is arranged in a series of discs which are connected to shaft, through the shaft the arranged discs are rotated [16]. The RBC equipment setup is require the following

components 1. Slow RPM motor, 2. Circular shape disks (the disks are made by foam material), 3. Ball bearings, 4. Shaft, 5. Semi-circular water tank (the volume of water tank is 6 to 8 liters). The above all elements are connected properly, the RBC equipment is equipped [16].

**Table 4: RBC equipment specifications [16]**

Name	Dimensions
Rotating speed	2 rpm
Diameter of the disk	20Ø cm
Surface area of disk	942.477 cm <sup>2</sup>
Submergence of the disk	45% of total surface area
Volume of the reactor	6 liters
Thickness of the disk	5 mm



**RESULT AND DISCUSSIONS**

**Dry weight Calculation of Biomass concentrations**

Mostly calculation of biomass is performed by number of count per one ml of solution or dry weight of one ml of solution. The calculation of biomass is most important because removal efficiency of treatment unit depends on microorganism concentration in aeration tank. The present work performed the *Staphylococcus aureus* and *E-coli* dry weight [9].

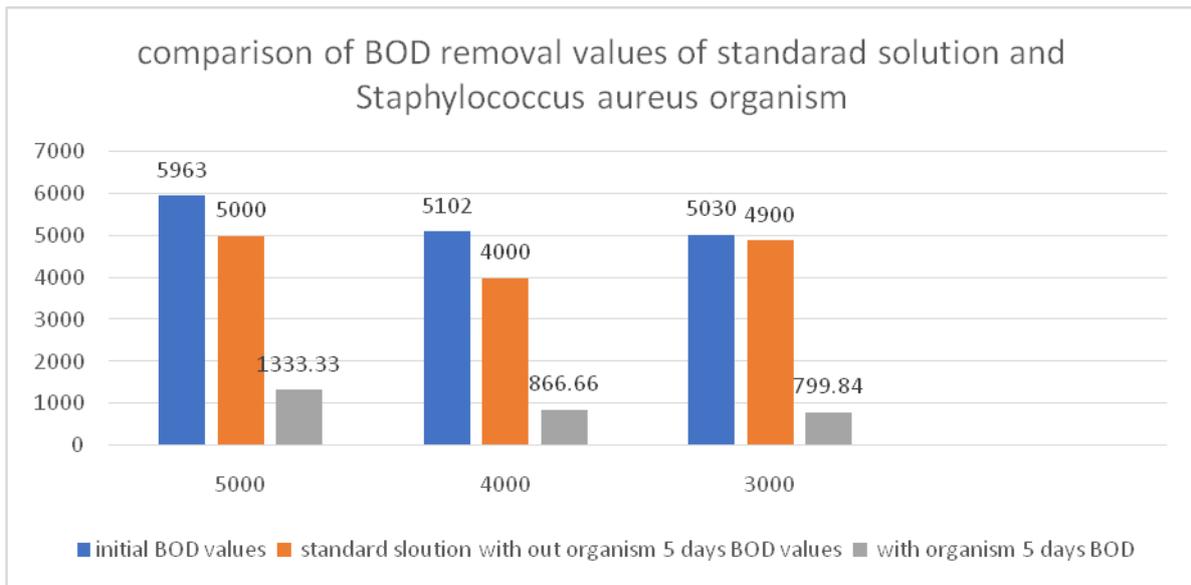
$$\text{Dry weight of Staphylococcus aureus} = \frac{[(\text{final weight of filter paper} - \text{initial weight of filter paper}) / (\text{volume of sample taken})] * 1000}$$

The dry weight of *Staphylococcus aureus* and *E-coli* are 6430 mg/l, 12270 mg/l.

**Batch study analysis**

The prepared synthetic dairy wastewater gives an approximate COD value of 17000 mg/l. This 17000 mg/l is diluted into three different concentrations as 5000 mg/l, 4000 mg/l and 3000mg/l. The Batch experiments are conducted at above mentioned concentration. At each concentration, BOD, COD and P<sup>H</sup> is evaluated. The batch experiments are used to find out efficiency of two different microorganisms. The two different microorganisms are *Staphylococcus aureus* and *Escherichia coli*. The batch experiments are conducted to find out high removal efficiency in *Staphylococcus aureus*. The *Staphylococcus aureus* are used to conduct rotating biological contractors (RBC).

**BOD removal efficiency by Staphylococcus aureus**



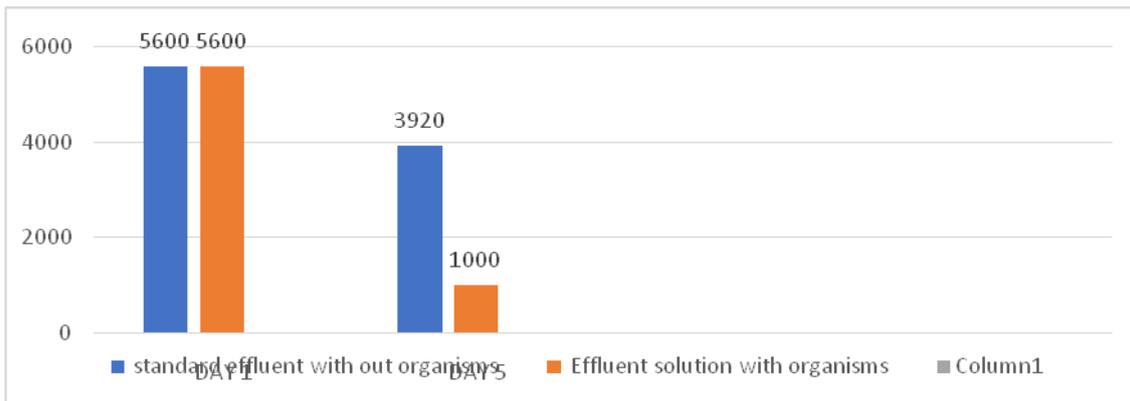
**Figure 1: BOD removal efficiency by Staphylococcus aureus**

The evaluated values are mentioned the above bar chart. The present experiment results are, at 5000 concentration, the naturally BOD is reduced to 5963 mg/l to 5000 mg/l of BOD it is represents in the form of percentage is 16.1495. The microorganisms added sample BOD removal is 5963 mg/l to 1333.33 mg/l of BOD. The removal efficiency at this stage is 77.63%. At 4000 concentration, the naturally BOD is reduced to 5102 mg/l to 4000 mg/l of BOD. At this stage the natural conditions reduce 21.59% of BOD. The microorganism’s added sample BOD removal is 5102 mg/l to 866.66 mg/l of BOD and the reduction of BOD is represents in the form of percentage is 83.013. At 3000 concentration, the naturally BOD is reduced to 5030 mg/l to 10000 mg/l of BOD. The microorganisms added sample BOD removal is 5030 mg/l to 2300 mg/l of BOD. At 3000 concentration the organisms gets 54.27%. Synthetic dairy water concentration increases at certain time period and decreases to zero due to natural conditions.

**Rotating biological contactors (RBC) batch studies**

The synthetic dairy wastewater is prepared based on table 1 and 2 proportions and kept 96 hours contact time. After 96 hours analysis of COD values 17000 ml/l, based on analysed COD values make dilutions to prepare 5000 ml/l of COD solution. The prepared 5000 mg/l of COD solutions is poured into RBC tank and also added 150 ml of Staphylococcus aureus solution. The present RBC is maintained constant of 2 RPM. The present condition is maintained 5 days. Before stating ending of RBC, BOD and COD values are analysed. Based on BOD values to find out the removal efficiency of Staphylococcus aureus in RBC batch studies. Similarly same concentration solution (standard solution) poured into 6 litters’ tank without RBC system and Staphylococcus aureus. The initial day BOD and 5<sup>TH</sup> day BOD is find out. The calculated values of standard effluent and treated effluent are used to find out alone removal efficiency of Staphylococcus aureus

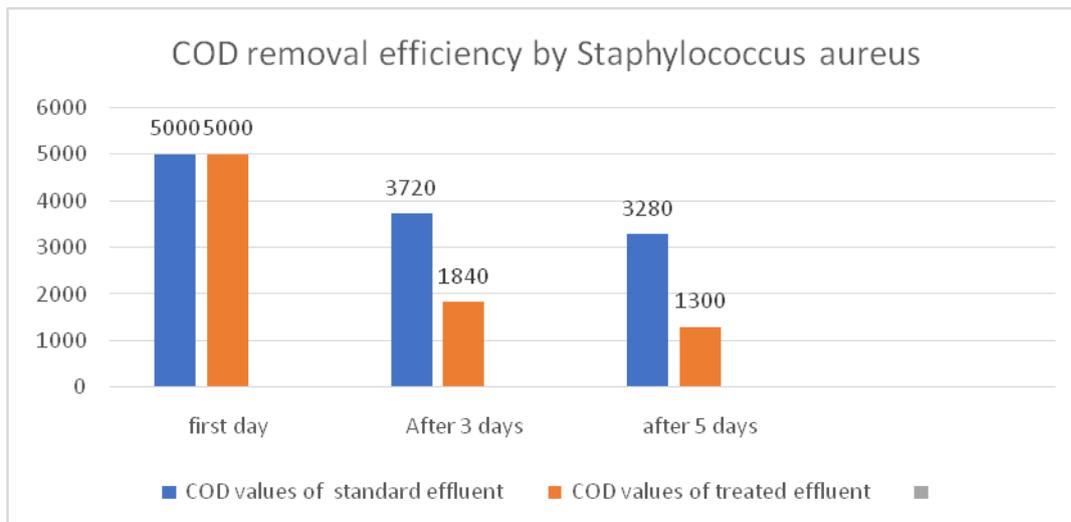
**BOD removal efficiency by Staphylococcus aureus in RBC batch studies**



**Figure 2: BOD removal efficiency by Staphylococcus aureus in RBC batch studies**

The graph represents BOD removal efficiency in biological treatment equipment of rotating biological contactors. The present work analysis know concentration of synthetic dairy waste water sample having a concentration of 5600mg/6litters of BOD. The graph shows two bars one is without organisms and second one is with organisms at specific conditions. The incubation period of 5 days is completed the treated sample is conducted BOD test. The BOD test results are presented above graph at DAY 5. The percentage of getting BOD removal efficiency in RBC is **82.14** at the same time naturally BOD removal efficiency is 30%.

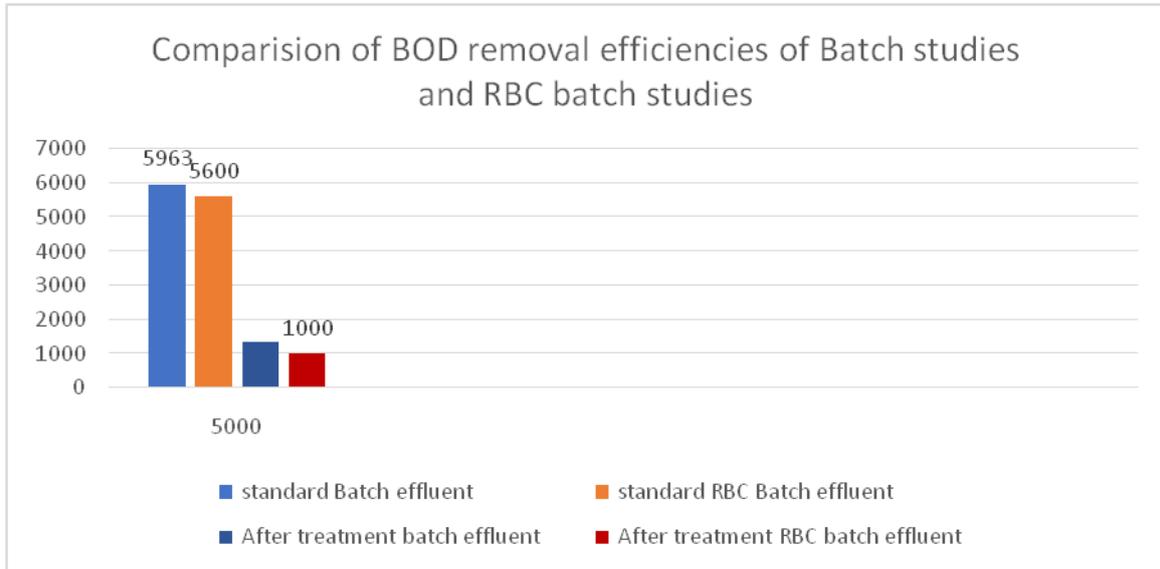
**COD removal efficiency by Staphylococcus aureus in RBC batch studies**



**Figure 3: COD removal efficiency by Staphylococcus aureus in RBC batch studies**

The represent graph is indicates COD removal efficiency in RBC at three different time periods. The analysis done by two know concentrations of different solutions means without organisms and with organisms. In the RBC sample the COD vales decreases from 5000 to 1840 at three days and 1300 mg/l at 5 days. The removal COD efficiencies of RBC at 3 days and 5 days are 63.2% and 74% respectively. The naturally removal efficiencies of COD at 3 and 5 days are 25.6 and 34.4.

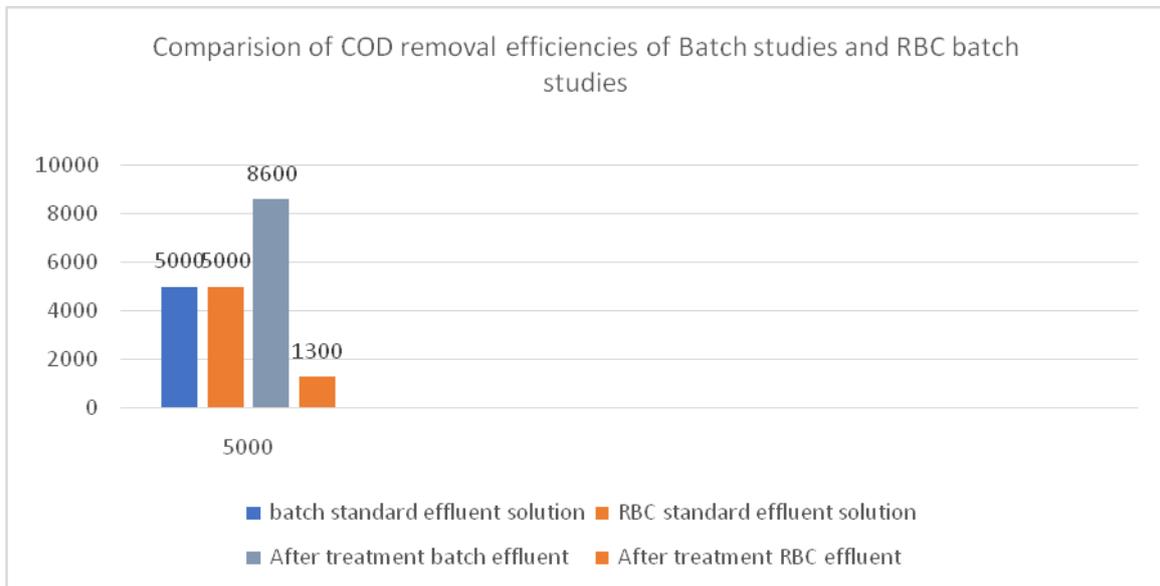
**Staphylococcus aureus BOD removal efficiency in synthetic dairy wastewater**



**Figure 4: Staphylococcus aureus BOD removal efficiency in synthetic dairy wastewater**

The present graph explains comparisons of BOD removal efficiencies in batch and RBC treatment units by *Staphylococcus aureus*. The removal efficiencies of batch and RBC are 77.6% and 82.142%. The graph represents removal efficiency is increases by providing support media to the organisms.

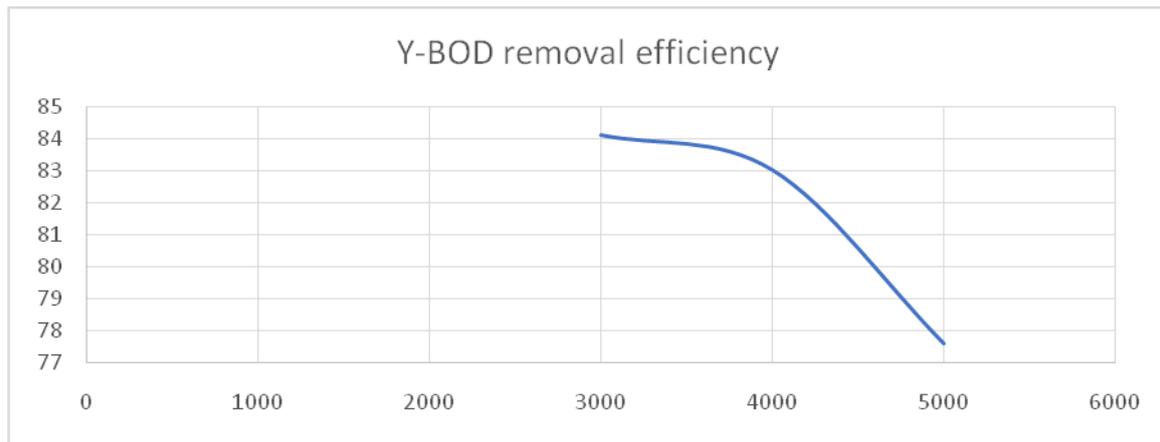
**Staphylococcus aureus COD removal efficiency in synthetic dairy wastewater**



**Figure 5: Staphylococcus aureus COD removal efficiency in synthetic dairy wastewater**

The present graph represents compression study of COD removal efficiency in batch and RBC by using *Staphylococcus aureus*. The above graph represent COD values slightly increases in batch studies due to the sample collection is from distributed sample at the same time COD values is decreasing in RBC because providing support media to the organisms and perfect sample collection. *Staphylococcus aureus* is effective in BOD and COD reduction in synthetic dairy wastewater.

**Comparison of concentration verses BOD removal efficiency in batch experiments**



**Figure 6: Comparison of concentration verses BOD removal efficiency in batch experiments**

The present graph represents BOD removal efficiency is increases with decreasing the concentration of the synthetic dairy wastewater

**CONCLUSION AND FUTURE SCOPE**

The existing project work involves treatment of synthetic dairy wastewater is prepared by using chemicals and milk powder and the composition of synthetic dairy wastewater is explained table number 1 and 2. The prepared synthetic dairy wastewater gets satisfactory concentration results corresponding to existing project work. The existing work is involves two microorganisms as developed from the laboratory and calculation of biomass concentration (dry density of biomass) as well as biomass sub-culturing is conducting in existing work. To get the expected results from one of the organism (*Staphylococcus aureus*) and another one (*Escherichia coli*) not get satisfactory results. The project work involves two equipment setups they are batch and RBC, the two equipment’s are developed in laboratory as prototype models. The developed two equipment’s are get satisfactory results in the present work. The results of present work is satisfactory to BOD removal efficiencies as decreasing the concentration of wastewater the removal efficiency is increasing by *Staphylococcus aureus* but COD removal is not satisfactory results are obtained. The COD values are slightly increases initial to final experimental results. The present work concludes *Staphylococcus aureus* is effective in removal of organic matter present in synthetic dairy wastewater. The BOD removal efficiency of *Staphylococcus aureus* in synthetic dairy wastewater is 84% and the COD removal efficiency is 74%. The one more conclusion is the microorganism growth is effective in attached system (RBC) compare to suspended system (batch). Based on the growth of microorganisms will decides the efficiency of biological treatment plant.

The executed experiment setup is used to calculate the growth kinetic of *Staphylococcus aureus*. The above mentioned results observation explains COD values increases up to certain time but region is not known excitedly. To find the region it requires some extension of research work. The RBC equipment setup is conducted at open atmosphere it achieves 84% removal efficiency but the RBC setup with *staphylococcus* are conducted in closed system it produces methane gas as end product because the used biomass is a facultative anaerobes.

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