# Performance Evaluation and Kinetic Study of Milk Processing Effluent in a Laboratory Scale Batch-fed Reactor

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**Abstract:** Dairy and milk processing units emanate huge quantity of liquid waste due to leaking from storage tanks, supply lines, vessels cleaning, cooling apparatus, pasteurizers, homogenizers etc. Effluents from milk processing units mainly contain organic compounds like oily matter, milk spills, lactose, whey etc. Biodegradation and Kinetic studies were carried out in a batch-fed reactor for evaluating the kinetic constants as necessary for design of a full scale bioreactor using suspended growth process. An investigation was done with simulated real life plant effluent as emitted from a nearby milk processing plant situated in West Bengal, India. After collecting wastewater from the above plant, the effluent wastewater was analysed in the laboratory and organic load (COD) was found in the range 1200-1300 mg/L whereas ammonia nitrogen ($NH_4^+$-N) varied in the range 45-55 mg/L, nitrate nitrogen was in the range 20-25 mg/L and Phosphorous ($PO_4^{3-}$-P) was in the range 20-25 mg/L. After acclimatization of the microbial seed in a laboratory, time concentration study of milk processing unit wastewater was conducted for reduction of  COD, ammonia Nitrogen ($NH_4^+$-N), Nitrate Nitrogen ($NO_3^-$-N) and phosphorous ($PO_4^{3-}$-P) separately in a batch-fed reactor. It was observed that the reduction of COD, $NH_4^+$-N, $NO_3^-$-N and $PO_4^{3-}$-P were in the range of 94-97%, 65-70%, 68-70% and 70-72% respectively. The kinetic constants such as K, $K_d$, Y and $K_s$ for the performed carbon oxidation, nitrification, denitrification and phosphorous removal under multi substrate component feeding condition were also estimated from the experimental results of batch study.

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## Introduction

Milk processing units are the centres where raw milk is processed, either for immediate consumption or converted into dairy product such as whey, cheese, butter, dried milk powder and ice cream. Milk processing units are classified as receiving, bottling, condensing, dry milk powder manufacturing, cheese making and butter making operation. These kind of milk processing units falls within the bulk organic polluting industries. Munavalli and Saler (2009) [1] reported about 2% of milk is wasted during the production process. Vourch et al. (2008)[2], mentioned milk handling plants emanate 0.2 to 10 L effluent on an average in every litre of milk to be processed. Different processing and operation units such as leaking tankers, spillage from storage tanks, cleaning operations release wastewater in a milk receiving establishment. So milk processing industries release huge amount of wastewater containing high BOD, COD, nutrients (Nitrogen and Phosphorous). Total nitrogen and phosphorus have the strength to affect aquatic life leading to eutrophication and algal blooms. Due to higher organic content, dairy wastewater is characterized by high BOD and COD varying from 0.1 to 100 g/L (Karadag et al. 2014, Dohare et al. 2014)[3,4]. Dairy effluent is reported to contain a BOD/COD ratio between 0.4-0.8 (Prazeres et al. 2012, Wang et al. 2009) [5,6] indicates highly biodegradable in nature. Slavov (2017)[7] stated whey, milk and permeates have high

COD load due to higher lactose concentration, which increases the soluble COD part, and biodegradable in nature. Vidal et al. (2000)[8],

stated that dairy and milk processing plants liquid waste are amenable for treatment with the help of both physico-chemical and biological methods. However, due to high chemical costs and associated sludge management issue in physical–chemical treatment processes, biological treatments are more preferred for removal of COD from dairy wastewaters. Apart from high COD load, dairy wastewater possesses, significant amount of $NH_4^+$-N, $NO_3^-$-N, $PO_4^{3-}$, which can be removed through biological treatment. Kargi and Dincer (1997)[9] recommended for biological treatment which is more effective to treat wastewater containing organic carbon and nutrients.

The present investigation was done for biological treatment of simulated milk processing unit effluent with acclimatized seeds and also to evaluate various kinetic co-efficient require for design of an appropriate suspended growth biological reactor.

## Materials and methods

**(A) Milk processing wastewater sample collection:**

Field wastewater sample was collected from a local milk processing unit locating near Sarisha, West Bengal. The milk processing plant generates wastewater with average flow rate of 90 $m^3$/day. The composite dairy wastewater samples were collected eight times in various season of a year during entire experimental study. Each sample was tested for parameters viz. pH, COD, $BOD_5$, TSS, TDS, $NH_4^+$- N $NO_3^-$-N and $PO_4^{3-}$-P following 'Standard Methods[10].
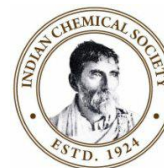
**(B) Seed acclimatization for organic carbon oxidation:**

The bacterial seed acclimatization study was performed in a vertical glass cylinder of 1L capacity. The acclimatized seed was cultured by inoculating 100 ml of non-acclimatized seed which was collected from nearby treatment plant and was mixed with 700 mL of distilled water along with 200mL of the stock feed solution which is prepared as per the mentioned constituents. The stock feed was prepared by adding 1.2 g/L milk powder, 0.5 g/L Dextrose, 0.25 g/L Beef extract, 0.25 g/L Yeast extract, 0.25 g/L peptone, 0.25 g/L Lactose. Necessary aeration in the reactor was accomplished with the help of mini compressor air pumps of 0.33hp capacity. pH in the feed solution was maintained in the range of 7.2-7.5. The seed acclimatization was continued for a period unless equilibrium state of removal in percentage of COD was achieved corresponding to a steady level of MLSS concentration and sludge volume index (SVI) with respect to an initial COD concentration as measured in the cylinder.

**(C) Seed acclimatization for nitrification:**

The seed acclimatization for nitrification was performed separately in a vertical glass cylinder of 1L capacity measuring cylinder. The acclimatized seed was cultured by inoculating 100 mL of non-acclimatized seed which was collected from nearby treatment plant. It was mixed with 700 mL of distilled water along with 200mL of the stock feed solution which was prepared as per the mentioned constituents. The stock solution was prepared with 0.2 g/L $NH_4Cl$, 0.1 g/L $(NH_4)_2SO_4$, 0.1 g/L Peptone, 0.3 g/L Dextrose, 0.25 g/L Beef extract, 0.25g/L Yeast extract. Aeration was done using aquarium pumps. pH was kept in the range of 7.2-7.5 by adding phosphate buffer. The Seeding was considered to be stabilized as steady performance

appeared with respect to consistent value of $NH_4^+$-N reduction corresponding to steady growth of MLSS concentration.

**(D) Seed acclimatization for denitrification:**

The seed acclimatization for denitrification study was performed in a 2L aspirator bottle which was provided with a magnetic stirrer. The acclimatized seed was cultured by inoculating 100 mL of non-acclimatized seed which was collected from an anaerobic digester and it was mixed with 700 mL of distilled water along with 200mL of the stock feed solution which was prepared as per the mentioned constituents. The stock was prepared with 0.01 g/L milk powder,0.01 g/L Dextrose, 0.05 g/L peptone, 0.05 g/L Beef extract, 0.05g/L Yeast extract, 0.01 g/L Lactose, 0.08 g/L $KNO_3$, 2ml Methanol. The feed solution was acclimatized under anoxic condition. Magnetic stirrer was provided to ensure proper mixing and to keep the sludge in the suspended condition. The acclimatization was performed in the anoxic condition for over three months.

**(E) Seed acclimatization for phosphate removal:**

The microbial seed acclimatization was performed in a 2L aspirator bottle which was provided with a magnetic stirrer and an aeration facility. The seed acclimatization of phosphorous utilizing organisms had been cultured by mixing anaerobically digested sludge of cow dung along with soil mud taken from a nearby wetland and small amount of sludge from the denitrifying reactor mixed with 1.5 L of distilled water in the aspirator bottle. The magnetic stirrer was provided to properly mix the sludge and keep the sludge in the suspended condition within the reactor. Alternate aeration facility was provided to supply oxygen during the aerobic phase. Alternate 24hours anoxic-aerobic condition was maintained in the aspirator bottle during seed acclimatization phase. The pH was
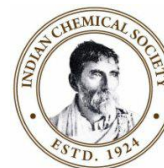
maintained at 7-7.5 with the help of carbonate solution (0.1 g $Na_2CO_3$) and this phase was continued for about three months.

## Experimental procedure

### (A) Batch carbon oxidation study:

The experiment for the batch carbon oxidation study was performed in a beaker of 1L which has been initially provided with aeration facility using aquarium pumps. For conducting the time concentration study for batch carbon oxidation 100 mL of the acclimatized sludge was used along with the synthetic milk processing wastewater. The first set (Set-1) of experiment was performed with initial COD concentration of 1289 mg/L and the second set (Set-2) of experiment was done for initial COD concentration of 1266 mg/L. Samples were taken time to time from reactor and analysed for the COD reduction along with the variation of pH and the increase concentration of MLSS.

### (B) Batch nitrification study:

The experiment for the batch nitrification study was performed in a beaker of 1L which has been initially provided with aeration facility using aquarium pumps. For conducting the time concentration study for batch nitrification 100 mL of the previously acclimatized nitrified sludge was used along with synthetic milk processing wastewater. The two sets of experiment (Set-1 and Set-2) were performed keeping an initial $NH_4^+$-N concentration of 55.4 and 47.2 mg/L, respectively. Samples were withdrawn in different time intervals and analysed thereafter for $NH_4^+$-N reduction along with variation of pH level and the increase concentration of MLSS within the reactor.

### (C) Batch denitrification study:

Time concentration study for batch denitrification conducted using 100 mL of the previously acclimatized denitrifier sludge with the prepared synthetic milk processing wastewater sample. The magnetic stirrer was provided to keep the sludge in suspended condition and for proper mixing of the sludge. The performance of denitrification study were done in two different initial $NO_3^-$-N concentration of 54.36 and 48.67 mg/L. Samples were collected at regular time intervals and analysed for $NO_3^-$-N reduction along with change of pH value and biomass growth (MLSS).

### (D) Batch phosphate removal study:

The experiment for the batch phosphorous removal study was performed in an aspirator bottle of 2L capacity which had been initially provided with a magnetic stirrer and aeration facility. Since the experiment was performed by altering the phases from anoxic to aerobic after definite intervals the aeration facility was provided and magnetic stirrer was also provided for properly mixing the sludge during the anaerobic phase. For conducting the time concentration study for batch phosphorous removal 100 mL of the previously acclimatized sludge has been used along with the prepared synthetic dairy wastewater sample. Two set of experiment was performed keeping the initial $PO_4^{3-}$-P concentration as 25.2 and 20.54 mg/L, respectively. Samples were withdrawn at certain predermined time interval from the reactor and analysed for residual $PO_4^{3-}$-P concentration.

## Results and discussions

### (A) Time concentration study for carbon oxidation:

Organic carbon oxidation was performed in the reactor for duration of 24hours with acclimatized seed. The COD was measured by taking samples time to time from the same reactor. Time-concentration graph for COD reduction in both set of experiment (Set-1 and Set-2) has been shown in Fig. 1 and Fig. 2 having initial COD concentration of 1289
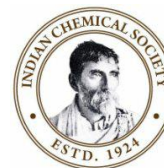
and 1266 mg/L to final COD concentration of 42 and 34 mg/L, respectively after 24 hours, corresponding to removal percentage of 96.74 and 97.31 respectively.
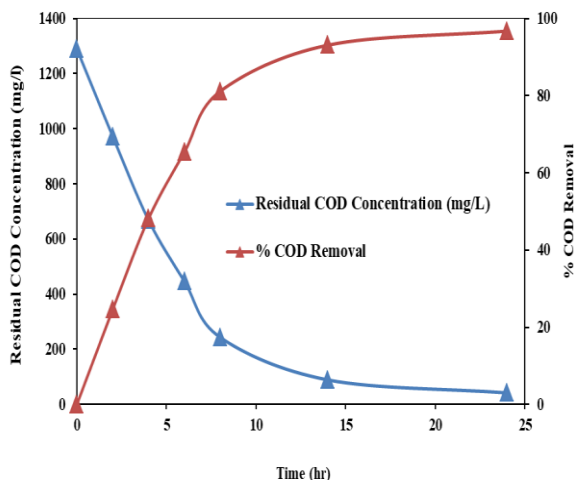
concentration was measured time to time by taking samples over the total react period. As shown in Fig. 3 and Fig. 4 the time vs. concentration graph, the $NH_4^+$-N concentration in both the setup decreases from an initial concentration of 55.4 mg/L and 47.2 mg/L to final concentration after 24 hours as 18.92 mg/L and 14.11 mg/L respectively, corresponding to removal percentage of 65.85 and 70.11 respectively.



**Fig. 1.** Carbon oxidation profile in batch reactor [initial COD = 1289 mg/L]
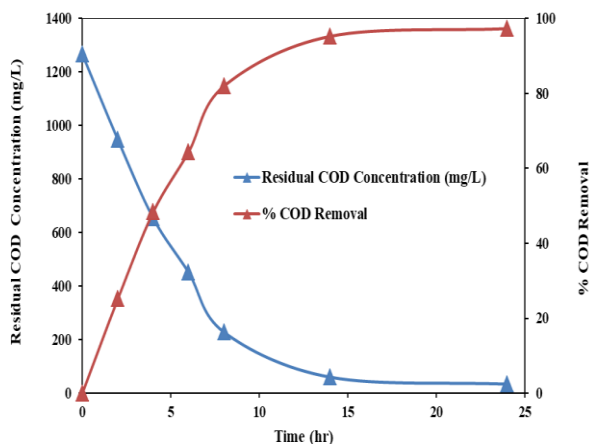


**Fig. 2.** Carbon oxidation profile in batch reactor [initial COD=1266 mg/L]

**(B) Time concentration study for nitrification**

The batch nitrification study was performed with acclimatized seed in the reactor for a period of 24 hours. The $NH_4^+$-N
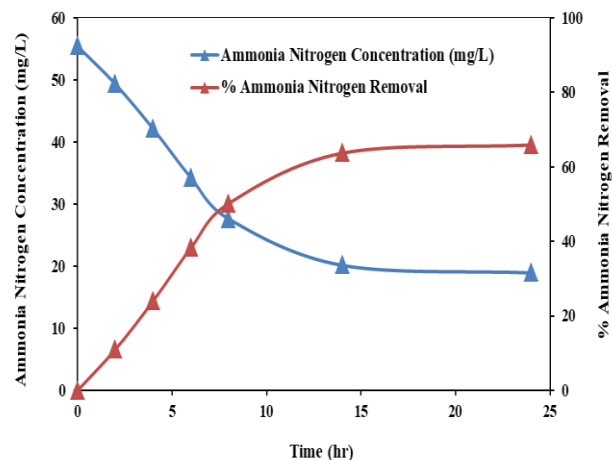


**Fig. 3.** Ammonia nitrogen removal profile in batch reactor [initial $NH_4^+$-N =55.4 mg/L]



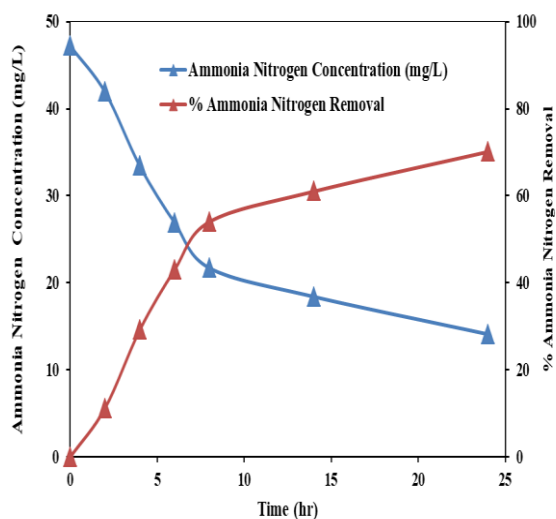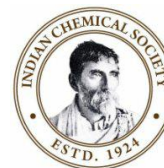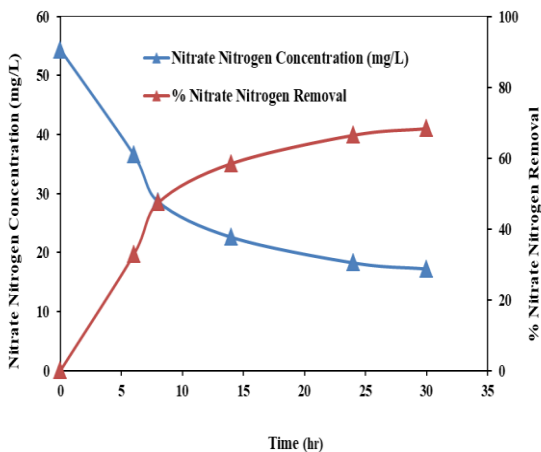**Fig. 4.** Ammonia nitrogen removal profile in batch reactor [initial$NH_4^+$-N= 47.2 mg/L]
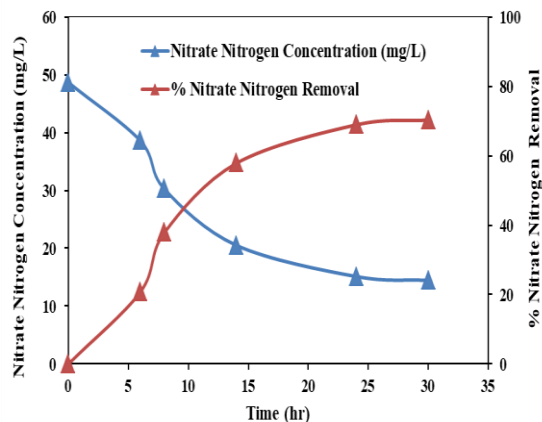
**(C) Time concentration study for denitrification:**

As per the experimental setup mentioned previously the time concentration study for the De-nitrification study was conducted. The batch study to observe the general trend of $NO_3^-$-N reduction was carried out with acclimatized seed in the reactor for a period up to 30hours. The $NO_3^-$-N was monitored at regular intervals by withdrawing samples over the total react period. As shown in Fig. 5 and Fig. 6 the time vs. concentration graph the $NO_3^-$-N concentration in both the setup decreases from an initial concentration of 54.36 mg/L and 48.67 mg/L to final concentration after 24hours as 17.21 mg/L and 14.45 mg/L respectively, corresponding to removal percentage of 68.36 and 70.31 respectively.



**Fig. 6.** Nitrate nitrogen removal profile in batch reactor [initial $NO_3^-$-N = 48.67 mg/L]

**(D) Time concentration study for phosphate removal:**

As per the experimental setup mentioned previously, the time concentration study for the phosphorous removal study was conducted. The batch study demonstrated the trend of $PO_4^{3-}$-P reduction. The $PO_4^{3-}$-P concentration was measured time to time by taking samples from the reactor. As shown in Fig. 7 the time vs. concentration graph, the $PO_4^{3-}$-P concentration in both the setup decreases from an initial concentration of 25.2 mg/L and 20.54 mg/L to final concentration after 24hours as 9.52 mg/L and 8.52 mg/L respectively, corresponding to removal percentage of 68.22 and 71.52 respectively.
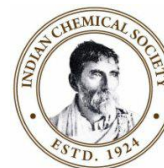


**Fig. 5.** Nitrate nitrogen removal profile in batch reactor [initial $NO_3^-$-N = 54.36 mg/L]
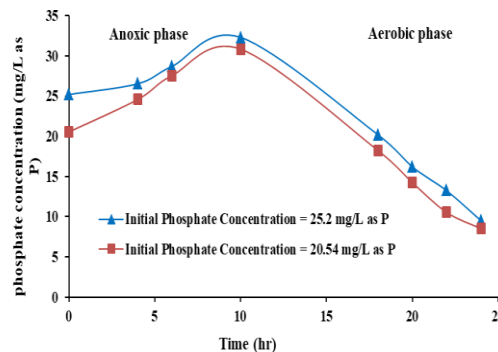
**Fig. 7.** Phosphorous removal profile in batch reactor

## Kinetic study for organic carbon removal, nitrification and denitrification

### (A) Kinetics for carbon oxidation:

A linear graph is shown in Fig. 8.The slope and intercept of best fit straight line is applied in equation $1/U_C = [(K_s/k)(1/S)]+1/k$ . The value of k is 1.77 per day whereas $K_s$ is 55.33 mg/L for carbon oxidation kinetic. The values of the reciprocal of the reaction time $(1/\theta)$ were again plotted with $U_C$ as shown in Fig. 9 for estimating the yield coefficient (Y) and decay coefficient $(k_d)$ by applying equation $1/\theta = YU_C - k_d$ . The value of Y is 0.642 mg of MLSS/mg of COD where as $k_d$ is 0.061per day.
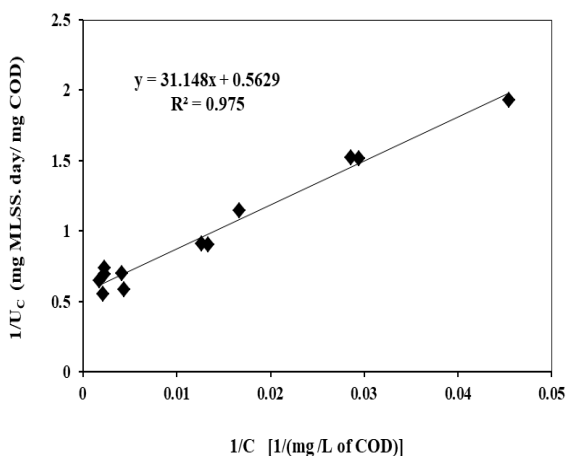


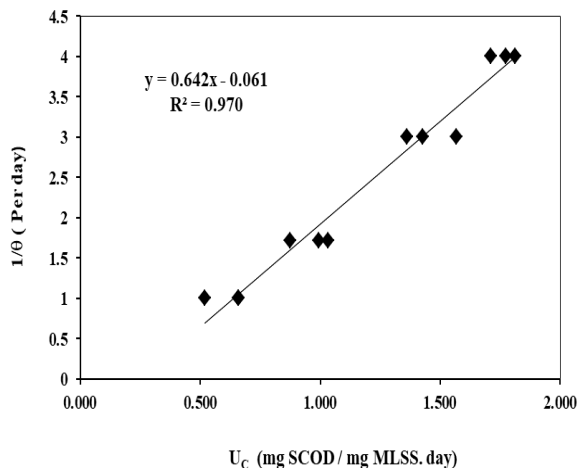**Fig. 8**. Graphical plot for substrate utilization kinetics for Carbon oxidation



**Fig. 9.** Graphical plot for microbial growth kinetics for carbon oxidation.

### (B) Kinetics for nitrification:

A best fit graph is plotted in Fig. 10. The slope and intercept of best fit straight line is applied in equation $1/U_N = [(K_s/k)(1/N)] + (1/k)$. The value of k is found to be 15.15 per day whereas the value of $K_s$ is about 27.57 mg/L the value of Y and $k_d$ for nitrification was obtained 0.565mg of MLSS/mg of $NH_4^+$-N and 0.046 per day, respectively as shown in Fig. 11.
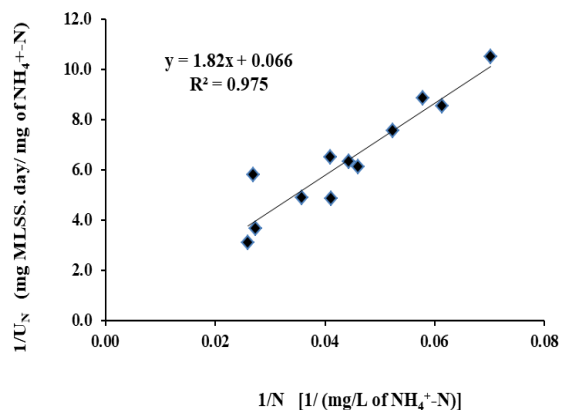


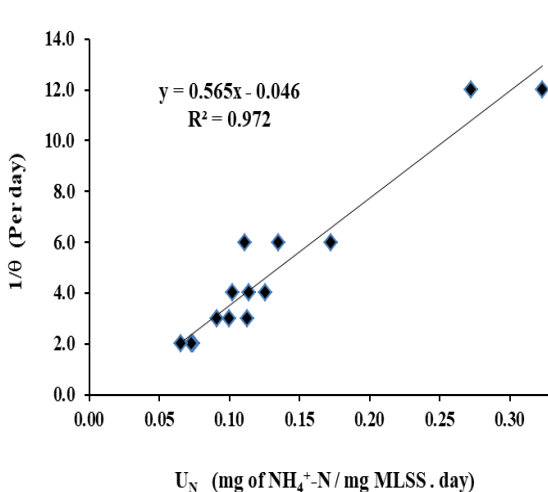**Fig. 10.** Graphical plot for substrate utilization kinetics for nitrification.
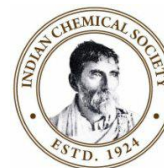
**Fig. 11.** Graphical plot for microbial growth kinetics for nitrification**.**



**Fig. 12.** Graphical plot for substrate utilization kinetics for denitrification

### (C) Kinetics for denitrification:

Kinetic study for Denitrification was done by an initial $NO_3^-$-N concentration of $50 \pm 2.5$ mg/L as N as shown in Fig. 12 and Fig. 13. The experimental values were plotted by using equation $1/U_{DN} = (K_s/k)(1/N') + 1/k$. The values of $1/\theta$ were plotted against $NO_3^-$-N utilization rate ($U_{DN}$) as shown in Fig. 12. The magnitude of k, $K_s$, Y and $k_d$ were found to be 1.37 per day**,** 11.98mg/L, 0.827 mg MLSS/mg $NO_3^-$-N and 0.063 per day, respectively. The values of kinetic coefficients of present study have been shown in Table-1.
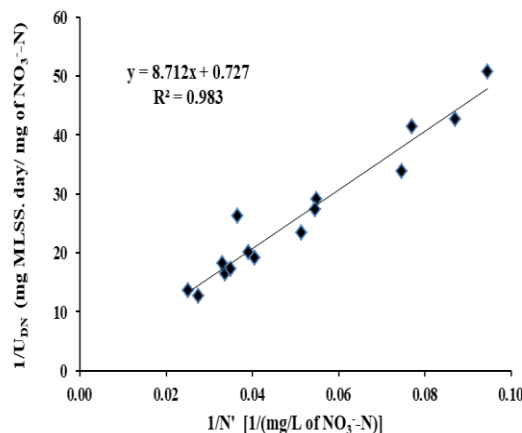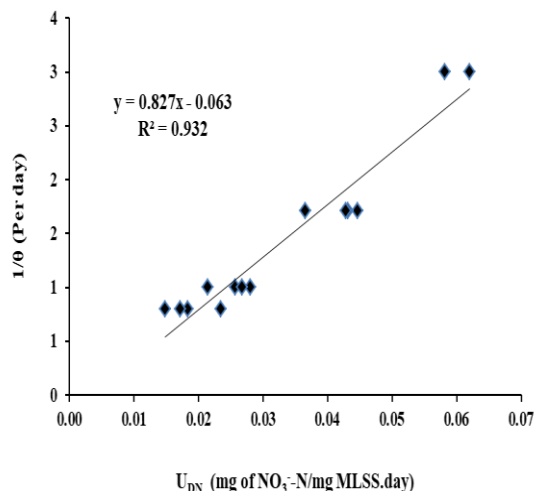


**Fig. 13.** Graphical plot for microbial growth kinetics for denitrification.

**Table 1.** Values of Kinetic Coefficients

| Sl No. | Kinetic coefficient | Carbon Oxidation | Nitrification | Denitrification |
|---|---|---|---|---|
| 1. | Y (mg MLSS/ mg of substrate) | 0.642 | 0.565 | 0.827 |
| 2. | $K_d$ (per day) | 0.061 | 0.046 | 0.063 |
| 3. | K (per day) | 1.77 | 15.15 | 1.37 |
| 4. | $K_s$ (mg/L) | 55.33 | 27.57 | 11.98 |

## Conclusion

The present research study has been carried out for the performance evaluation and kinetic study of a laboratory scale batch-fed reactor for treating the effluent of a milk processing unit. This study has been conducted for the biological removal of COD, ammonia Nitrogen, Nitrate Nitrogen and Phosphorous. The time-concentration study depicts a removal percentage of 95-97 for COD, 65-70 ammonia nitrogen, 68-70 nitrate nitrogen and 70-72 phosphorous is achievable. Based on these experiments conducted kinetic coefficients has also been evaluated and are found to be corroborating with the findings of other researchers.

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