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Excess cell mass as an internal carbon source for biological denitrification

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ABSTRACT

Aim of the present work was to examine whether the SCOD (soluble chemical oxygen demand) released after the physical disruption of excess activated sludge can be used as an alternative carbon source for biological denitrification. In the first stage of research, we investigated the potential use of energy efficient hydrodynamic cavitation (HC) technique for the disruption of activated sludge. In a comparative study between ultrasonic cavitation (UC) and HC, it was observed that UC needs five times more energy than that of HC to release the same amount of SCOD. In the second stage of the experimental study, SCOD was successfully used as an alternative carbon source (alternative to sodium acetate) for biological denitrification. The critical weight ratio ($SCOD/NO_3$–N) of seven ensured 100% removal of nitrate. Nitrate removal kinetics indicated that denitrification with SCOD as a carbon source gives higher specific denitrification rate (by ≈200%) as compared to conventional carbon source (sodium acetate).

1. Introduction

Activated sludge treatment is the most widely used technique for the treatment of industrial liquid waste. This approach often results in large quantity of sludge formation as the byproduct, which needs to be treated and disposed off as per the prescribed norms. Treatment cost of excess of sludge, i.e., drying and incineration is about 60% of overall treatment cost of wastewater treatment plant (WWTP) (Saby et al., 2002).

Activated sludge disruption is carried out either by chemical disruption or by mechanical disruption. Chemical lysis includes chlorination and ozonation, while physical disruption includes ultrasonic cavitation (UC) and high speed or high pressure homogenization induced hydrodynamic cavitation (Zhang et al., 2007; Show et al., 2007; Save et al., 1994). Amongst chemical methods, ozone is not a selective oxidant and ozonation is relatively expensive (Liu, 2003). Chlorination is an option for chemical lysis. However, chlorination produces undesirable chlorinated by-products such as trihalomethane (THM) and results in poor sludge settleability (Saby et al., 2002; Park, 2001).

UC is one of the approaches used for the physical disruption of activated sludge. The mechanism of cell disruption in UC can be either by shock wave generated by collapse of a cavity (Save et al., 1994) or due to shear stress generated by dynamic oscillation of cavities (Doulah, 1977). Recently, Mahulkar et al. (2008) have developed a mathematical model for quantifying the stress generated by the collapsing and/or oscillating cavity based on the dynamics of the cavity. Similar to Doulah (1977) it is hypothesized that when the stress generated by the cavity oscillation and/or collapse exceeds the strength of a microbial cell wall, the cell is disrupted.

Many investigators have reported the use of UC for sludge disruption (Zhang et al., 2007; Show et al., 2007) but acoustic cavitation is relatively expensive technique having limited scalability. In this article hydrodynamic cavitation technique (HC) has been shown to be an alternative technique for the partial disruption of activated sludge from the wastewater treatment plant. In HC technique, cavitation is produced by pressure variation, obtained using velocity variation created by variable area geometry. For example, based on the geometry of system, the interchange of pressure and kinetic energy can be achieved resulting in the generation of cavities as in the case of flow through orifice, venturi, etc. The mechanism of cell disruption is likely to be similar in both UC and HC. Rate of cell disruption depends on the intensity of cavitational collapse and the cell wall strength and in case of HC; intensity depends on the cavitation number (C_v) defined as follows:

$$C_v = \frac{(P_1 - P_v)}{(1/2\rho u^2)} \quad (1)$$

where 'P_1' is atmospheric or downstream pressure (Pa), 'P_v' is vapour pressure of the medium at the operating temperature, 'ρ' is density of sludge (kg/m^3) and u is the velocity of sludge through the throat (m/s) of the cavitation device, a venturi in this case. For

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C_v = 1.0, cavitational inception occurs and with further reduction in C_v the cavitational collapse intensity and the number of cavitational events increase.

As specified earlier, treatment and disposal of excess activated sludge requires nearly 60% of total effluent treatment cost. According to MacDonald (1990), the cost of the required carbon contributes as high as 70% of the total denitrification cost. Disruption of microbial cell releases intra-cellular organic matter, which can be expressed in terms of (soluble chemical oxygen demand) SCOD and these intra-cellular organic matter can possibly be used as an alternative carbon source for biological denitrification, thus reducing the overall cost of denitrification as well as addressing the issue of disposal of excess activated sludge.

The nitrate wastes, generated by industries and excessive use of fertilizers have raised alarming levels of nitrate in the ground water (up to 1800 mg/l of NO_3–N) in some part of India (Majumdar and Gupta, 2000). Nitrogen in its oxidized form causes potential health hazard to humans and also causes eutrophication of the natural still water. So, treatment and disposal of nitrate waste has become a major concern.

Biological denitrification is the most widely used option and has proved to be an economically feasible technique for complete removal of nitrogen from its oxidized form, i.e., nitrate and nitrite. The general equation describing biological denitrification is as follows (Walker et al., 1989):

$$NO_3^- + \text{Carbon Source} \xrightarrow{\text{Microorganism}} N_2 + CO_2 + H_2O + OH^- + \text{Cell} \quad (2)$$

Several commercially available chemicals have been used as carbon source for denitrification. Commonly used carbon source are methanol, ethanol, acetic acid, acetate, methane, etc. (Akunna et al., 1993; Abu-Ghararah, 1996; Dhamole et al., 2007; Biradar et al., 2008; Rajapakse and Scutt, 1999). Degradation of nitrate takes place in five consecutive steps but generally it is considered that nitrate is first converted to nitrite (rate limiting steps) and then to nitrogen gas. Zero order kinetic equation for nitrate and nitrite degradation is represented as follows as suggested by (Moore and Schroeder, 1971; Foglar et al., 2005; Dhamole et al., 2007)

$$NO_3 \xrightarrow{K_1} NO_2 \xrightarrow{K_2} N_2 \quad (3)$$

$$\left(\frac{dC_{NO_3}}{dt}\right) = -K_1 \quad (4)$$

$$\left(\frac{dC_{NO_2}}{dt}\right) = K_1 - K_2' \qquad \text{In presence of nitrate} \quad (5)$$

$$\left(\frac{dC_{NO_2}}{dt}\right) = -K_2 \qquad \text{In absence of nitrate} \quad (6)$$

$$K_1^1 = \frac{K_1}{X} \text{ and } K_2^1 = \frac{K_2}{X} \quad (7)$$

where C_{NO_3}, C_{NO_2} and X are concentration of nitrate, nitrite and biomass concentration MLSS (g/l) and K_1 is zero order rate constant for degradation of nitrate. K_2 and K_2' are zero order rate constants for degradation of nitrite in presence and in absence of nitrate, respectively. K_1^1 and K_2^1 are specific rate of nitrate and nitrite reduction. Values of rate constants and specific degradation rate can be found elsewhere (Foglar et al., 2005; Dhamole et al., 2007).

This article examines energy efficient way of excess sludge disruption (HC) and use of SCOD released from sludge disruption process as a sole carbon source for nitrate removal in biological denitrification process. The study also delineates optimization of the required SCOD/NO_3–N ratio, nitrate and nitrite removal kinetics with sodium acetate, SCOD individually as well as combined SCOD and sodium acetate as carbon source.

2. Methods

2.1. Ultrasonic and hydrodynamic cavitation assisted activated sludge disruption

An ultrasound generator (Sonics, USA) with ultrasound frequency of 20 kHz, net power (delivered) of 45 W, diameter of 1 cm and 4 cm depth of submergence of horn was used for activated sludge disruption process. Activated sludge sample with initial volume of 200 ml and MLSS concentration of 30 g/l was taken in a jacketed container with a means of temperature control using cold water. Temperature of the sample was maintained at 27 ± 2 °C. Disrupted samples were collected at different time intervals for SCOD analysis.

A HC setup comprising of reservoir tank, centrifugal pump (2700 rpm, 373 W) and cavitating venturi in a closed loop setup was used to carry out the experiment. Four liters of activated sludge with initial biomass concentration of 30 g/l was taken in the tank. Biomass was circulated through a cavitating venturi at 3 atm discharge pressure of pump, delivering at a flow rate of 14 l/min. Measured velocity of sludge through venturi was 18.55 m/s and cavitation number was observed to be 0.549 (calculated from Eq. (1)). Flow rate of effluent in the set up can be managed by using the bypass line. Process was continued for 60 min and SCOD released was measured at regular time intervals by collecting the sample from the reservoir.

2.2. Denitrification with conventional carbon source (sodium acetate)

A pre-adapted consortium from high strength nitrate waste treatment process (Biradar et al., 2008) with initial concentration of 6 g/l was used for the comparative study. The experiments were performed in 1 l sequential batch reactor with 0.4 l working volume. Four bladed pitch blade impeller with ½ h.p. overhead motor with 100–200 rpm was used to keep the biomass in uniform suspension in the reactor. Sodium acetate was added with C/NO_3–N ratio (by weight) of 2.33. Reaction was carried out in 24 h cycle, in which 22 h was the total reaction time, 1.5 h for settling and 0.5 h for decanting and refilling (200 ml of feed). After settling, biomass was thoroughly washed with de-ionized water to remove residual COD (unconsumed sodium acetate) from the reactor.

Initial composition of medium for this experiment was $NaNO_3$ = 3.95 g/l, CH_3COONa = 3.78 g/l, Na_2HPO_4 = 7 g/l, K_2HPO_4 = 1.5 g/l in 0.2 l of de-ionized water.

2.3. Denitrification with SCOD

Same experimental setup was used to investigate the use of SCOD as a carbon source. After disruption of sludge in HC process, it was centrifuged at 10,000 rpm (18,650*g*) for 30 min and clear supernatant was decanted. The supernatant having SCOD of 2332 mg/l was made up to 6 l by diluting with the de-ionized water. SCOD of 1515 mg/l was obtained after the dilution and was stored in a cold room at 4 °C. Stock SCOD sample was used as a carbon source to carry out denitrification study and optimization of SCOD/NO_3–N ratio (by weight). SCOD/NO_3–N ratio was varied by changing the nitrate concentration in the medium. No other carbon compounds or nutrients were added in the medium. Change in nitrate and nitrite concentration was measured at regular time intervals. Moreover, MLSS, nitrate, nitrite and SCOD concentrations were measured every day before decanting the treated effluent from the reactor.

2.4. Denitrification with SCOD and conventional carbon source (blend study)

Denitrification with SCOD/NO_3–N ratio of 6 was incomplete and remaining unconverted 117 mg/l of nitrite in the reactor was denitrified by adding sodium acetate as a carbon source with C/NO_3–N ratio of 2.33 based on the earlier results (Dhamole et al., 2007; Biradar et al., 2008). Complete denitrification was achieved with this supplementary sodium acetate.

3. Analytical method

Performance of the reactor was monitored by analyzing samples for nitrate, nitrite, SCOD and mixed liquor suspended solid (MLSS) after completion of 22 h reaction time. Liquid samples were centrifuged at 10,000 rpm (5585*g*) in the microcentrifuge (Tarsons Spinwin Microcentrifuge MC-01). Supernatant obtained was used for the analysis of nitrate and nitrite using DIONEX Ion chromatograph, AS11 (2 mm) column and guard column. NaOH (12 mM) was used as an eluent. SCOD was measured with potassium dichromate reflux method. Prior to the analysis of SCOD, samples were centrifuged at 10,000 rpm (5585*g*) and filtered with 0.2 μm membrane filter (Make: Sartorious, Minisart). Dry weight of biomass was expressed in terms of MLSS and was determined following standard methods (APHA et al., 1992).

4. Results and discussion

4.1. Ultrasonic and hydrodynamic cavitation assisted activated sludge disruption

Activated sludge from fertilizer industry with initial MLSS of 30 g/l was used in both UC and HC assisted microbial cell disruption study. Sludge disruption was quantified in terms of continuous release of intra-cellular organic matter (which was measured in terms of SCOD release with time). In the case of UC, SCOD concentration was increase gradually with respect to time. In 60 min of sonication, 4319 mg/l of SCOD was released (Fig. 1). However, in case of HC process, fast release of intra-cellular organic matter was observed in initial 10 min with SCOD concentration reaching a value of 2213 mg/l and only a marginal further increase in SCOD was seen (increase in only 119 mg/l) in the remaining 50 min of treatment. SCOD released in initial 10 min was significantly high as compared to UC (SCOD concentration of 1238 mg/l in the initial 10 min under UC as against 2213 mg/l in HC).

Though there is no linear increase in the SCOD concentration, but initial impact of HC was relatively high enough (slope during initial 10 min was 221 mg/W h in HC, while in UC slope of initial 10 min was 124 mg/W h). In a HC setup with flow rate of 14 l/min and 18.55 m/s velocity, equilibrium in SCOD concentration (limiting disruption) was reached in 13 passes of 4 l of sludge through the cavitating venturi. SCOD profile with energy delivered is as shown in Fig. 2. Comparing the SCOD release of 2213 mg/l in both cases, UC requires 76 W h, while HC needs just 15 W h. Thus, UC needs five times more energy than that for HC to achieve same quantity of SCOD release. Based on the measured energy consumption it was observed that cost per kg cost of SCOD release in case of HC is less than the cost of commercial grade sodium acetate. The asymptotic SCOD value obtained in the case of HC can be explained on the basis of the cavity dynamics. The cell disruption occurs when the shear stress generated by oscillating/collapsing cavity exceeds the cell wall strength. The operating conditions of HC have a certain limiting value of the shear stress depending on the cavity dynamics, controlled by the operating condition and hence no further cell disruption occurs. SCOD release can be further increased by either further decreasing the cavitation number (i.e., increasing the velocity of the sludge through the venturi) or by using different cavitational device such as an orifice or any other mechanical constriction of different geometry. SCOD release can be further increased by using hybrid cavitation technique (HC coupled with UC). Recent, study by Kampas et al. (2009), has shown the use of disrupted activated sludge as a carbon source for biological nutrient removal. However, the method used for the disruption of activated sludge, was not economical compared to cost of conventional carbon source. HC assisted disruption process thus could be used to extract intra-cellular organic matter with less amount of energy.

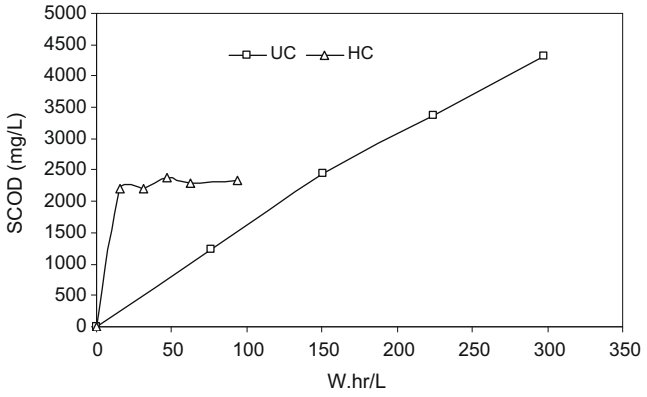

Fig. 2. SCOD release profile with respect to energy delivered in case of UC and HC with biomass concentration of 30 g/l.

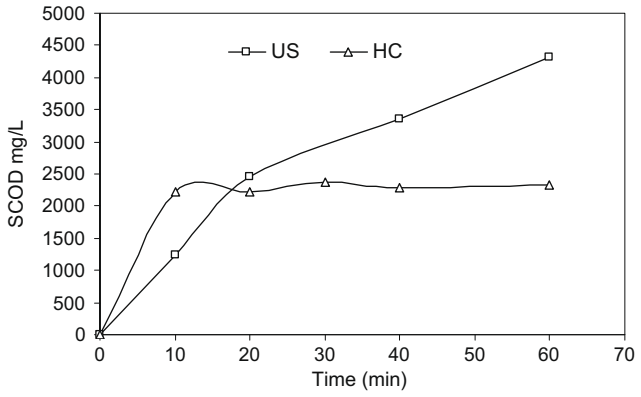

Fig. 1. SCOD release profile with respect to time in case of UC and HC with biomass concentration of 30 g/l.

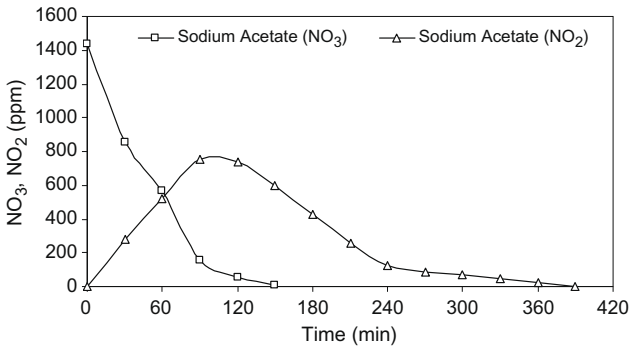

Fig. 3. Nitrate and nitrite reduction profile during denitrification of 1440 mg/l of NO_3 with sodium acetate, C/NO_3–N ratio (wt.) of 2.33 and biomass concentration of 6 g/l.

4.2. Denitrification with conventional carbon source (sodium acetate)

Biomass adapted with synthetic waste in four stages (Biradar et al., 2008) was used for this study. Initial nitrate concentration in feed was 1440 mg/l of NO_3 and initial biomass concentration was 6 g/l. C/NO_3–N weight ratio of 2.33 was maintained by adding sodium acetate as a carbon source. Complete removal of nitrate was achieved in 150 min, while accumulated nitrite during the reaction was removed in a total time of 390 min (as shown in Fig. 3). Kinetics of nitrate and nitrite degradation reaction follows zero order (Dhamole et al., 2007; Biradar et al., 2008). Kinetic parameters such as rate constants and specific denitrification rate for nitrate and nitrite were calculated using Eqs. (4)–(7). From the kinetic rate constant values (as shown in Table 1) it appears that the specific nitrate removal rate K_1^1 with sodium acetate as a carbon source is relatively lower (for example, $K_1^1 = 138$ mgNO_3/g MLSS/h vs. 354 mg NO_3/g MLSS/h in case of SCOD/NO_3–N of 10) than all cases of SCOD used. In case of specific nitrite removal rate K_2^1 is also lower (for example, $K_2^1 = 42.5$ mgNO_2/g MLSS/h vs. 147.6 mg NO_2/g MLSS/h in case of SCOD/NO_3–N of 10) than the specific nitrite removal rate with sodium acetate as a carbon source. Reason for lower rate of nitrate and nitrite reduction with sodium acetate is explained in the next section (denitrification with SCOD).

4.3. Denitrification with SCOD

Denitrification study was continued with the same biomass which was used for denitrification with sodium acetate as a carbon source. Study was carried out to investigate the optimum SCOD/NO_3–N ratio. Stock sample (6 l) with initial SCOD of 1515 mg/l was stored and used as a carbon source feed for sequential batch reactor. SCOD/NO_3–N ratio was varied by varying the nitrate concentration in the feed. Denitrification study was carried out initially with the SCOD/NO_3–N ratio of 10 and biomass concentration was (MLSS) 2 g/l. Complete removal of nitrate and nitrite was achieved in just one hour with maximum nitrite accumulation of 97.1 mg/l in first 30 min and ratio of actual SCOD consumed to NO_3–N removed was observed to be 4.17 (shown in Table 2). Actual SCOD consumed to NO_3–N removed was calculated by analyzing SCOD remaining in the reactor after complete removal of nitrate and nitrite. In the next step SCOD/NO_3–N ratio was reduced to 8 (from 10) by increasing the nitrate concentration in the feed. Complete removal of 419.4 mg/l of NO_3 was achieved in 80 min with MLSS of 3, maximum nitrite peak of 229.3 mg/l was observed during the first 30 min of reaction time with an increase in the ratio of actual SCOD consumed to NO_3–N removed, to 5 (from 4.17) was observed. Nitrate removed with respect to time for SCOD/NO_3–N ratio of 10 and 8 is as shown in Fig. 4. Kinetic rate constant values calculated for different SCOD/NO_3–N ratio are as shown in Table 1. When, SCOD/NO_3–N ratio was further reduced to 7, nitrate removal of 479.3 mg/l was achieved in 45 min with maximum nitrite accumulation of 278.6 mg/l, which was removed in 35 min of further treatment. Increase in the ratio of actual SCOD consumed to NO_3–N removed was also measured (i.e., to 7 from 4.17 and 5 for SCOD/NO_3–N ratio of 10 and 8, respectively) and with no SCOD remaining in the reactor after the complete removal of nitrate and nitrite. In the last step, when SCOD/NO_3–N ratio was further decreased to 6, maximum nitrite peak (accumulation of nitrite in reactor) was observed in 45 min with concentration of 303.8 mg/l, with complete removal of 559.2 mg/l of nitrate. The accumulated nitrite was reduced to 117 mg/l from the initial peak of 303.8 mg/l in 90 min of further treatment but no further decrease in the nitrite concentration was observed (76% of total nitrogen removal efficiency was observed) for the next 195 min of reaction time, indicating a possible limitation of the available carbon source for metabolism. Profile of nitrate and nitrite degradation at SCOD/NO_3–N ratio of 7 and 6 is as shown in Fig. 5. Incomplete denitrification at SCOD/NO_3–N ratio of 6 could be

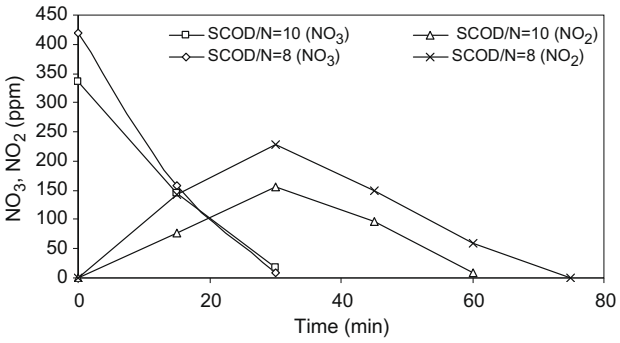

Fig. 4. Nitrate and nitrite profile during denitrification of 335.5 mg/l and 419.4 mg/l of NO_3 with SCOD as a carbon source, SCOD/NO_3–N ratio (wt.) of 10 and 8 and biomass concentration of 2 and 3 g/l.

Table 1
Zero order denitrification rate of nitrate and nitrite at different SCOD/NO_3–N ratio and with sodium acetate (K_1 = rate of nitrate reduction, K_2' = rate of nitrite reduction in presence of nitrate and K_2 = rate of nitrite reduction in absence of nitrate) and K_1^1 and K_2^1 are specific zero order denitrification rate of nitrate and nitrite reduction.

SCOD/NO_3–N or C/NO_3–N ratio	Initial nitrate concentration in feed (mg/l)	K_1 (mg NO_3/l/h)	K_2' (mg NO_2/l/h)	K_2 (mg NO_2/l/h)	MLSS (g/l)	K_1^1 (mg NO_3/g MLSS/h)	K_2^1 (mg NO_2/g MLSS/h)
C/N = 2.33 (with sodium acetate)	1440	828.5	316.8	254.8	6	138.1	42.5
SCOD/N = 10	335.5	708.0	395.2	295.3	2.0	354.0	147.6
SCOD/N = 8	419.4	819.7	337.9	310.6	3.0	273.2	103.5
SCOD/N = 7	479.3	895.1	317.2	337.7	3.5	255.7	96.5
SCOD/N = 6	559.2	767.8	347.8	162.0	4.5	170.6	36.0

Table 2
Initial nitrate concentration, maximum nitrite peak observed during the reaction and ratio of SCOD consumed to NO_3–N removed at different SCOD/N ratio in feed.

SCOD/NO_3–N in Feed	Initial nitrate concentration in feed (mg/l)	Maximum nitrite accumulation (mg/l)	MLSS (g/l)	$SCOD_{consumed}$ (mg/l)	$SCOD_{comsumed}/NO_3\text{–}N_{removed}$
10.00	335.50	97.07	2.0	315.90	4.17
8.00	419.39	229.35	3.0	473.30	5.00
7.00	479.30	278.56	3.5	757.60	7.00

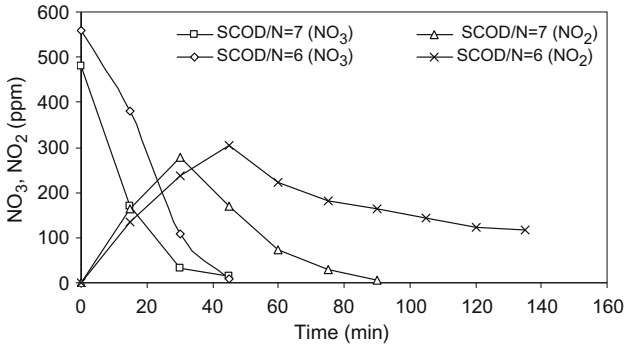

Fig. 5. Nitrate and nitrite profile during denitrification of 479.3 mg/l and 559.2 mg/l of NO_3 with SCOD as a carbon source, SCOD/NO_3–N ratio (wt.) of 7 and 6 and biomass concentration of 3.5 and 4.5 g/l.

attributed due to exhaustion of electron donor (carbon source) for the subsequent reduction of accumulated nitrite.

During the study, nitrite peak was found to be increasing with an increase in nitrate concentration in the feed. The reason behind the accumulation of nitrite has been discussed by Dhamole et al. (2007). The sludge which was classified in two type, i.e., nitrate respirator (converts nitrate to nitrite) and true denitrifier (converts nitrate to nitrogen gas). Increase in the concentration of nitrate respirator and preference of nitrate over nitrite as electron acceptor can lead to the accumulation of nitrite in the reactor. Moreover, during the investigation of optimization of SCOD/NO_3–N ratio, actual SCOD consumed to NO_3–N removed was increasing with increasing the initial nitrate concentration in the feed. Since biomass concentration increased from 2 g/l to 4.5 g/l, necessiting an increasing carbon consumption for cell maintenance and cell formation (values of actual SCOD are shown in Table 2).

From the kinetic analysis it was observed that denitrification with SCOD as a carbon source gives higher denitrification rate as compared to conventional carbon source (sodium acetate). The reason behind this could be the type of carbon source, which plays an important role in denitrification (carbon is the rate limiting substrate in biological denitrification processes). Intra-cellular organic matter comprises different natural organic compounds, which can be used as a carbon source and can be easily metabolized, the rate of denitrification is expected to be more. Specific degradation rate of nitrate in all the cases using SCOD as a sole carbon source studied was higher than the specific denitrification rate with sodium acetate (as shown in Table 1). The specific degradation rate of nitrite was also higher in all cases except at SCOD/NO_3–N ratio of 6. The decreasing trend of specific nitrite removal rate (from SCOD/NO_3–N ratio of 10, 8, 7 and 6) is possibly due to non-availability of carbon source (early consumption and exhaustion) in the reactor.

4.4. Denitrification with SCOD and conventional carbon source (blend study)

In this stage of the investigation, denitrification of remaining quantity of accumulated nitrite during the denitrification of SCOD/NO_3–N ratio of 6 (i.e., 117 mg/l of NO_2 remaining in the reaction) was treated by adding sodium acetate to the reaction mixture with C/N ratio of 2.33. Complete removal of remaining nitrite was achieved in further 90 min after the addition of this supplementary carbon source. From this experiment it can be conclusively said that SCOD can also be simultaneously used as a supplementary carbon source for biological denitrification process. Excess activated sludge from the effluent treatment plant can be treated with cavitation and intra-cellular organic matter released from the cell can be used as an alternative/supplementary carbon source for biological denitrification.

5. Conclusion

Studies on excess activated sludge disruption with HC indicate the possibility of the released SCOD as a carbon source for biological denitrification process. SCOD released by HC process can be further improved. High initial release of SCOD with HC indicates that, it can be coupled with the UC to achieve higher SCOD release (hybrid cavitation) to reduce further the energy required. The studies with SCOD as a carbon source for biological denitrification with critical SCOD/NO_3–N ratio of 7 have been found. The blend study confirms that the HC can be coupled with the high strength nitrate waste treatment plants readily and the cost of the required carbon source can be significantly reduced. The rate constants with SCOD as a carbon source were found to be higher than synthetic carbon source.

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