TREATMENT OF WASTEWATERCONTAINING CARBOHYDRATES USINGPICHIA SAITOI CULTURE

T. Amani

Biochemical and Bioenvironmental Engineering Research Center, Sharif University of Technology Tehran, Iran

S. Yaghmaei and V. Maghsoodi

Department of Chemical and Petroleum Engineering, Sharif University of Technology Tehran, Iran, yaghmaei@mehr.sharif.edu

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Abstract Treatment of wastewater containing carbohydrates by *Pichia saitoi* growing on beet molasses was investigated in a well-mixed continuous tank as an alternative to bulking control.The yeast strain that used in this work was isolated from non-alcoholic beverage industrial wastewater, with a view on TOC removal compared with other strains in previous study.In this research the isolated yeast showed high COD and TOC removal at three hydraulic retention times, HRT= 48, 24 and 18 hours.Maximum COD and TOC reductions were obtained at HRT=48 hours, which were 96% and 88%, respectively.The influent COD and TOC were 2500 and 148 mg/l, respectively.The pH maintained for synthetic wastewater was about 9.The changes of pH within the aeration tank at each HRT was monitored.Effects of organic loading rate (OLR) on COD and TOC reduction efficiencies were studied in this work.Aeration rate was between 0.2-0.25 vvm, which was very low in comparison to the other studies.This yeast strain had high settling ability (maximum SVI was observed at 60 ml/g) and also high F/M ratios (maximum of F/M was 2.9 kg COD/kg MLSS.day).

Key Words Bulking, Carbohydrates, Pichia Saitoi, Activated Sludge

چکیده دراین تحقیق تصفیه بیولوژیکی پساب مصنوعی ملاس چغندر قند بوسیله کشت سوش مخمری در یک تانک هوادهی اختلاط کامل، به منظور کنترل توده ای شدن لجن مورد بررسی قرار گرفت.سوش مخمری که مورد استفاده قرار گرفت، از پساب کارخانه نوشابه سازی جداسازی شده، که از نظر حذف TOC با سایر سوشهای جداشده از این پساب در تحقیق قبلی مقایسه شده بود.در این تحقیق، توانایی این سوش در حذف پساب آلی در سه زمان ماند هیدرولیکی ۴۸، ۲۴و۱۸ ساعت ارزیابی شد.حداکثر راندمان بدست آمده در زمان ماند ۴۸ ساعت مشاهده شد، که برای COD و TOC به ترتیب برابر ۹۶ و۸۸ درصد بود.تغییرات pH در تانک هوادهی با توجه به اینکه pH پساب مصنوعی در حدود ۹ تنظیم شده بود، اندازه گیری شد.اثر تغییرات بار آلی بر روی راندمان حذف COD و TOC در این تحقیق مطالعه شد.سرعت هوادهی بین vvm ۰/۲۵–۰/۲ تنظیم شد،که این مقدار در مقایسه با سیستمهای دیگر بسیار پایین می باشد.از جمله حصوصیات با ارزش این سوش مخمری، قابلیت ته نشینی بالا(حداکثر مقدار SVI برابر ۶۰ میلی لیتر بر گرم)و همچنین نسبت بسیار بالای F/M (حداکثر مقدار F/M برابر kg COD/kg MLSS.day ۲/۹) می باشد.

1. INTRODUCTION

Activated sludge is commonly used process in the secondary treatment of wastewater containing biodegradable organic materials [1]. The activated sludge is a suspended cell growth process, which caution is needed in the daily operations. In the activated sludge process there are often problems with sludge return, sludge disposal and bulking [2-4]. Bulking of activated sludge is a phenomenon when mixed culture has low settling rates. The settling and thickening characteristics of activated sludge are judged in practice according to the sludge volume index (SVI) [5]. There are two types of bulking: non-filamentous and filamentous bulking. The much worse case and more undesirable bulking is the filamentous bulking produced by excessive growth of filamentous microorganisms. Filamentous microorganisms are present in any activated sludge. Bulking problems appear when the filamentous grow faster and overrun the flocformers. Overgrowing of filamentous in activated sludge is affected mainly by: composition of treated wastewater, actual concentration of dissolved oxygen in aeration tank, actual concentration of soluble substrate under which microorganism grow, degree of accumulation capacity regeneration and technological parameters of the process. The most frequent causes of filamentous bulking raised by wastewater composition are; higher contents of saccharide and sulfides, nutrient deficiency (N,P) and low pH[5].In this study, effects of wastewater composition on bulking was investigated. So high concentration of sugars (such as, sucrose) was used in synthetic wastewater. In practice such condition occasionally occurs in non-alcoholic beverage industrial wastewater. In a conventional activated sludge plant (with MLSS<3500 mg/l) the normal range of SVI must be 50-150 ml/g [6].

Filamentous bulking can be controlled by means of chemicals and a natural selection of non-filamentous microorganisms [5]. In this study, latter method was used, so a yeast strain, which was isolated from non-alcoholic beverage industrial wastewater, was cultivated in the well-mixed aeration tank on beet molasses as the synthetic wastewater. The beet molasses contains more than 50% sugar (such as, sucrose), which is a good subtrate to use by yeasts. Sugars can cause bulking phenomena. Pollution is quantified by the total organic carbon (TOC) and chemical oxygen demand (COD). The claims of this study was inspection of settleability of the yeast to control the bulking phenomena and so other cases such as effects of inhibitors and DO was not inspected.

2. MATERIALS AND METHODS

2.1 Experimental Unit A simplified schematic diagram of the experimental setup is shown in Figure 1.Bioreactor was a 28.8 l aeration tank. Sintered glass diffusers inserted in the sidewalls provided air supply in tank. The diffusers not only supplied the oxygen necessary for the respiration of the microorganism (above 2 mg/l, aeration flow rate was 0.2-0.25 vvm) but also provided turbulent mixing of the liquid to ensure that adequate substrate and oxygen were available throughout the vessel. The wastewater temperature was controlled at 25-30 ^{0}C throughout the aeration tank. Residence time distribution (RTD) studies were carried out to analyze the hydrodynamic behavior of aeration tank by adding a pulse of inert tracer to tank under the normal operating conditions [7].

When an impulse of tracer was added under normal operating conditions tracer immediately appeared in the output upstream of tank at its peak value and then decayed away exponentially. Therefore, this tank could be considered a complete mixed type [7]. The pH of feed (synthetic wastewater, diluted beet molasses, pH about 7) was adjusted about 9 by sodium hydroxide solution (1 normality). The unit was operated as a continuous tank reactor, which was fed by a peristaltic pump.

2.2 Wastewater In order to have wastewater containing carbohydrates with uniform physical and chemical characteristics throughout the experimental studies, synthetic wastewater was prepared by dissolving known amount of beet molasses as carbon source in tap water. To provide balanced food for the growth of microorganism responsible for the biodegradation of organic matter in the wastewater, ammonium phosphate and urea were added in appropriate quantities to get 100:5:1.Volume of feed on the base of flow rate was prepared and flow rate values according to the HRTs (48,24,18 hr) were selected, which were 10, 20 and 26.67 ml/min. So the feed was prepared on daily bases.

2.3 Microorganism The yeast strain was ***Pichia saitoi CBC 49110*** (Figure 2) provided by department of Chemical and petroleum Engineering,

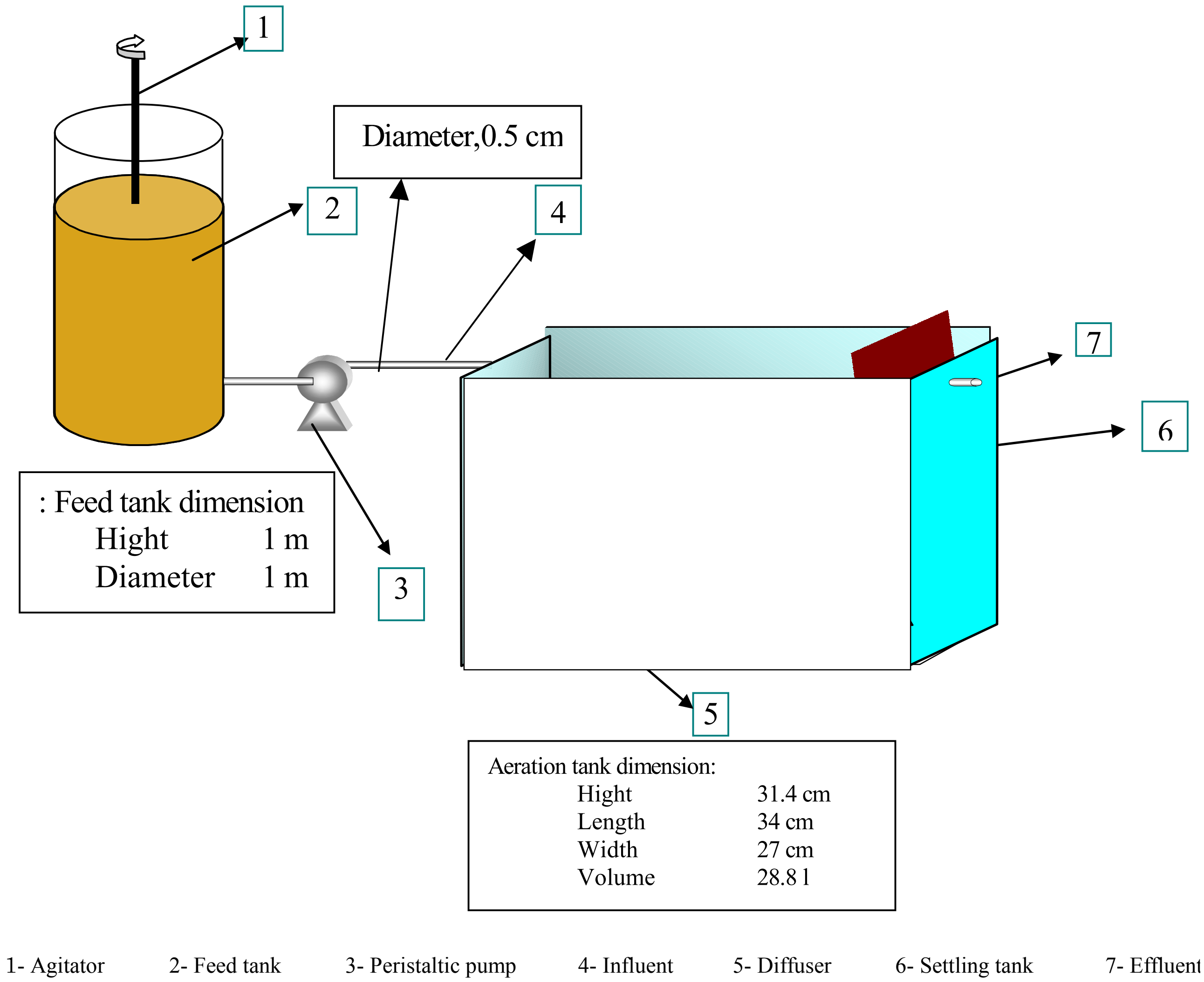


Figure 1. Schematic presentation of experimental unit.

Biochemical and Bioenvironmental Engineering Research Center, Sharif University of Technology, Iran [8]. ***Pichia saitoi CBC 49110*** was identified taxonomical, and compared with other strains in the viewpoint of TOC removal this strain was selected for our work. This yeast has maximum activity to removal of TOC at high pH, and this was an exception among the yeasts [8]

2.4 Preparation First the yeast strain was cultivated in slant culture on PDA medium and incubated at 30 °C for 48 hours and then cultivated in preculture as a inoculum. The preculture medium was Sabor Dextrose Broth. Inocula were provided in 500 ml Erlenmeyer flasks, containing 150 ml growth medium and placed on an orbital shaker (130 rpm) in a thermo stated room (30 ^{0}C) for 48 hours. The carbon and nitrogen sources were, glucose 20 g/l and peptone 10 g/l, respectively. Inoculation to the aeration tank was 5% by the volume.

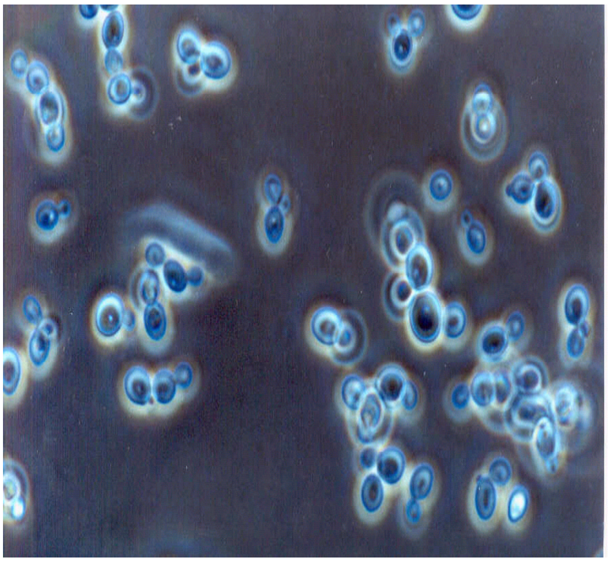


Figure 2. Pichia saitoi CBC 49110 in budding.The photo was taken by electronic microscope, in Biochemical and Bioenvironmental Research Center, Chemical and Petroleum Engineering, Sharif University of Technology.

2.5 Kinetic Constants Of Flow Reactor Studies It is necessary to evaluate kinetic parameters for simulating wastewater treatment processes [9].

The flow reactor has been widely used for kinetic studies. In the case of Monod kinetics in a completely mixed reactor under the steady-state condition, the material balance becomes [1]:

$$\frac{\theta X}{S_0 - S} = \frac{K_S Y}{\mu_m}\left(\frac{1}{S}\right) + \frac{Y}{\mu_m} \quad (1)$$

$$\frac{S_0 - S}{X} = \frac{K_d \theta}{Y} + \frac{1}{Y} \quad (2)$$

In this way, at several steady-state hydraulic retention times (HRT), θ within the reactor the inlet and outlet substrate and biomass concentration (S_0, S, X) were measured. By plotting $\frac{\theta X}{S_0 - S}$ versus $\frac{1}{S}$, in Equation 1 the slope and intercept are $\frac{K_S Y}{\mu_m}, \frac{Y}{\mu_m}$ and by plotting $\frac{S_0 - S}{X}$ versus θ, in equation 2 the slope and intercept are $\frac{K_d}{Y}, \frac{1}{Y}$. Therefore, the four kinetic parameters required for monitoring the yeast growth, K_S, K_d, μ_m and Y are obtained.

3.ANALYTICAL METHODS

Organic content was determined by the total organic carbon (TOC) concentration, which was measured with a Skalar-TOC Analyzer-CA 10, and the chemical oxygen demand (COD) concentration by potassium dichromate method. Biomass concentration was evaluated by mixed liquor suspended solids (MLSS) concentration, which is determined in conformity with Standard Methods. Also, sludge volume index (SVI) was measured by the standard method [10].

4.RESULTS AND DISCUSSION

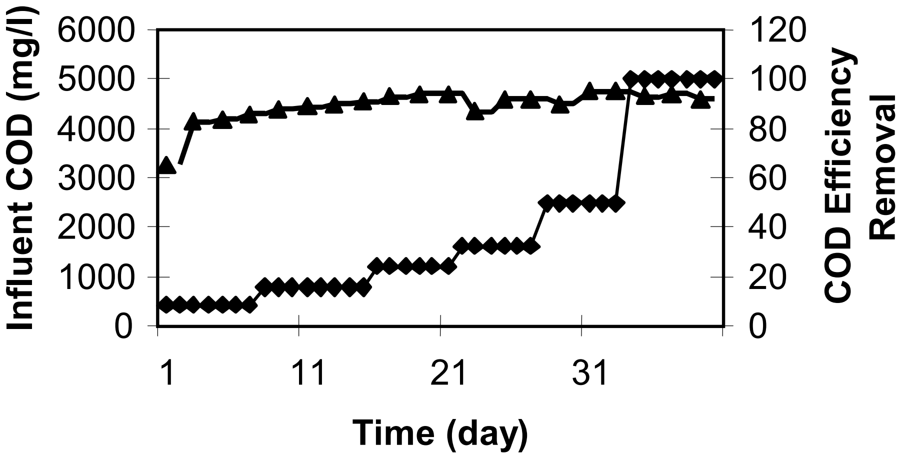
It is known that the sludge bulking often occurs under increased organic loading rates in the activated sludge process, especially when the wastewater contains a large quantity of carbohydrates. In this study, in order to investigate the treatment capacity of the yeast culture in aeration tank, the organic loading was gradually increased during the experiments at three HRTs, 48, 24 and 18 hours (Figures 3, 4, 5). Maximum

COD removal was obtained at HRT= 48 hours, which was 95% at influent COD= 5000 mg/l. Upper organic loading rate (OLR) was limited on the basis of typical COD of non-alcoholic beverage industry wastewater. Maximum OLR was used at HRT= 24 hours, which was 5 gCOD/l.day and COD removal was 86% at it.

According to OLR, HRT and COD removal efficiencies, led to conclusion that optimum HRT was 24 hours. Maximum efficiency was 92% at OLR= 2.5 g COD/l. day.

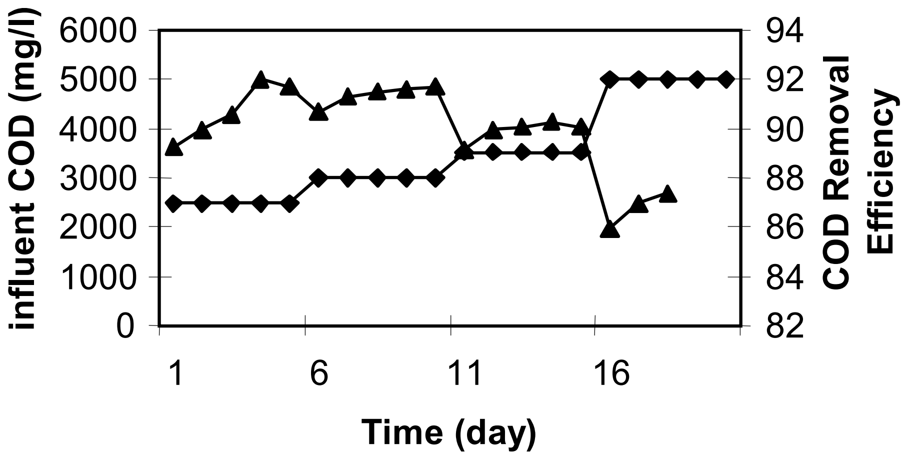
TOC efficiency was analogous to COD removal efficiency. TOC inlet and removal efficiencies are shown in Figures 6, 7 and 8.

Kinetic parameters as mentioned above were



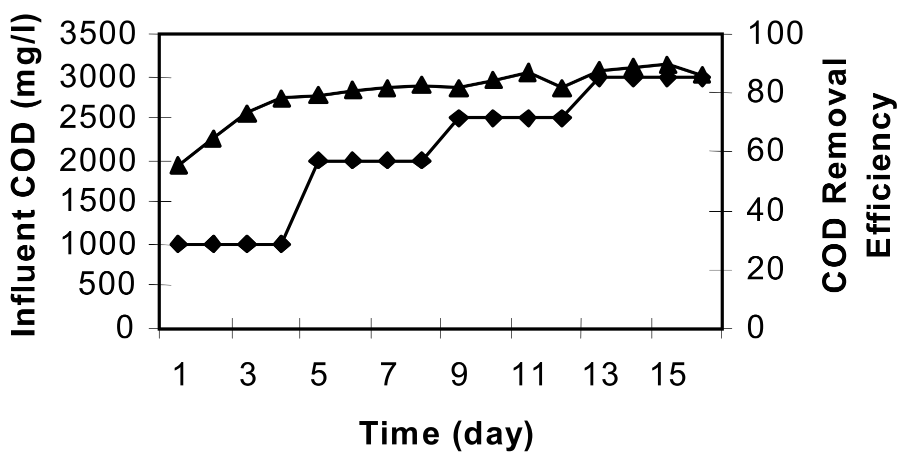
▲: COD efficiency; ♦: COD influent.

Figure 3. Increasing COD loading and COD removal efficiency at HRT = 48 hr.



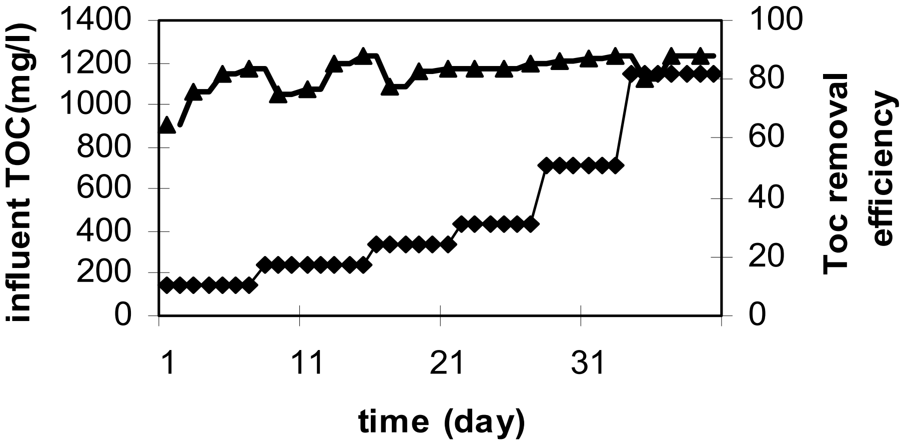
▲: COD efficiency ; ♦:COD influent.

Figure 4. Increasing COD loading and COD removal efficiency at HRT= 24 hr.



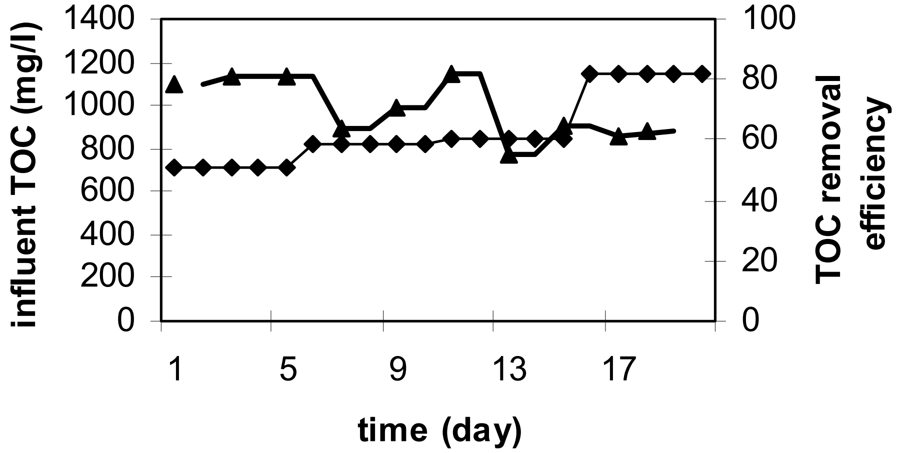
▲: COD efficiency; ♦: COD influent.

Figure 5. Increasing COD loading and COD removal efficiency at HRT= 18 hr.



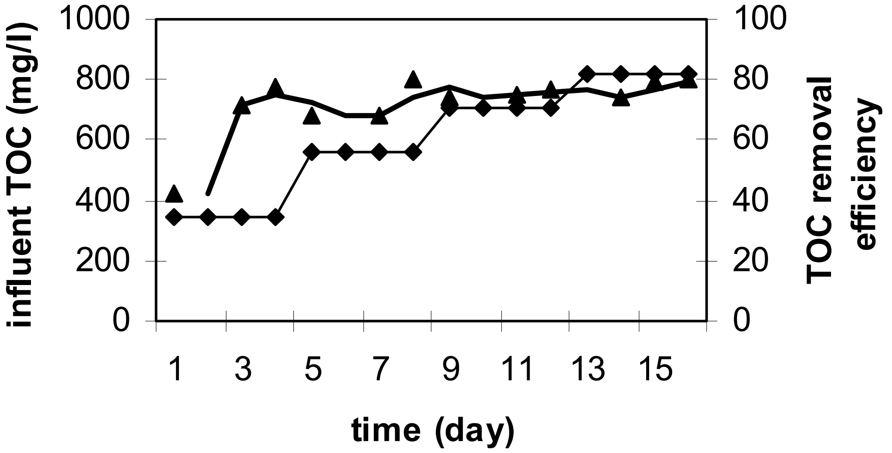
▲: TOC efficiency; ♦: influent TOC.

Figure 6. TOC inlet and removable efficiency at HRT = 48 hr



▲: TOC efficiency; ♦: influent TOC.

Figure 7. TOC inlet and removal efficiency at HRT= 24 hr.



▲: TOC efficiency; ♦: influent TOC.

Figure 8. TOC inlet and removal efficiency at HRT= 18 hr.

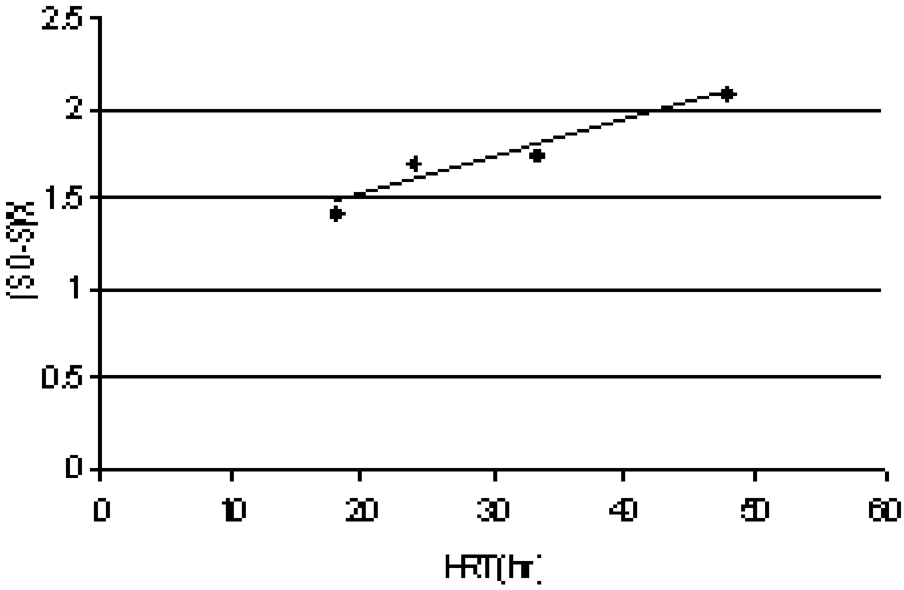


Figure 9. Estimation of kinetic parameters, Equation 2 (K_d, Y), $\theta = HRT$.

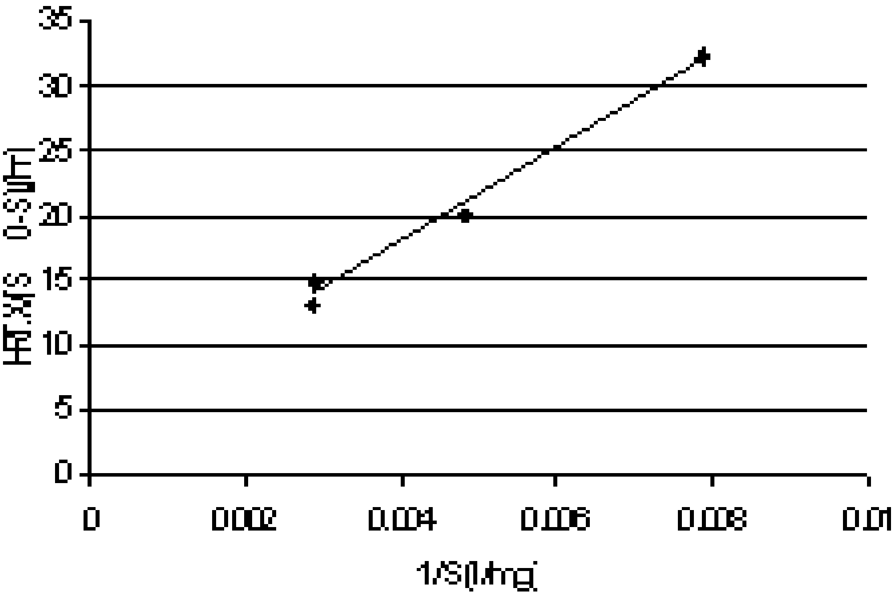
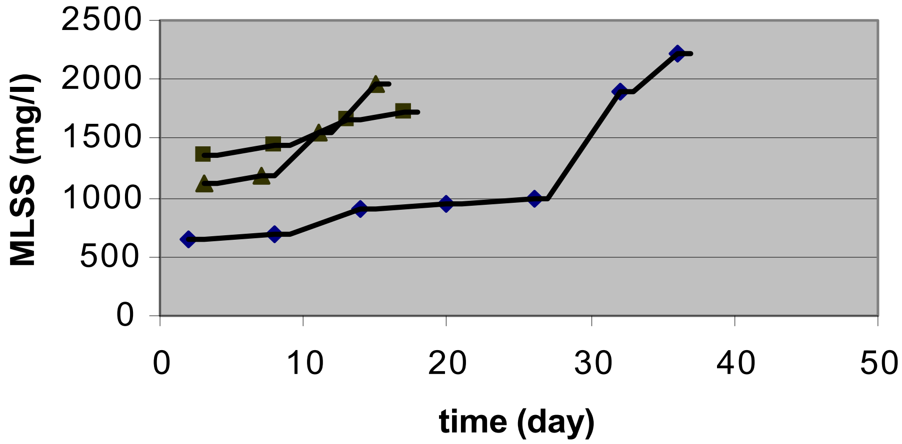


Figure 10. Estimation of kinetic parameters, Equation1 (K_S, μ_m), $\theta = HRT$.

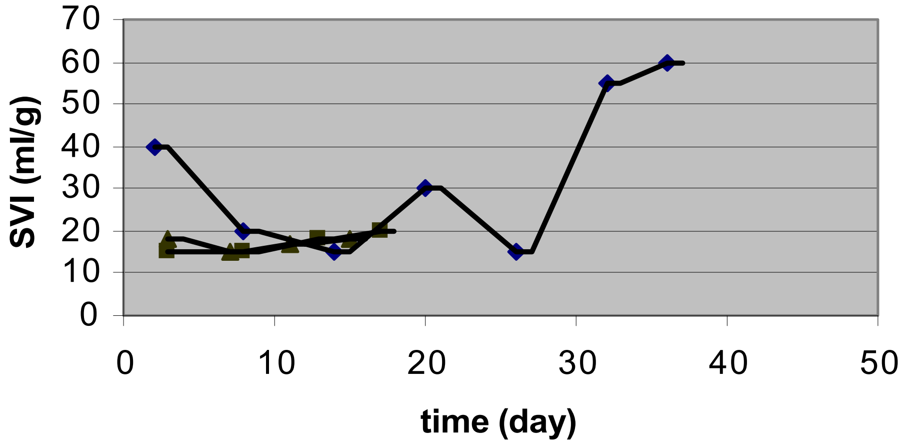
obtained, which are $K_d = 0.018\,hr^{-1}$, $Y = 0.895$ (Figure 9), $\mu_m = 0.263\,hr^{-1}$ and $K_S = 1075\,mg/l$ (Figure 10).

Figure 11, shows mixed liquor suspended solids (MLSS) values versus time for three HRTs. MLSS is a measure of biomass concentration in this system. Maximum value of MLSS was obtained at



♦: HRT= 48 hr; ■: HRT= 24 hr; ▲: HRT= 18 hr.

Figure 11. MLSS concentration versus time for each HRT.



♦: HRT= 48 hr; ■: HRT= 24 hr; ▲: HRT= 18 hr.

Figure 12. Sludge volume index (SVI) versus time for each HRT.

HRT= 48 hours, which was 2220 mg/l.

Low aeration rate was used for all experiments to reduce energy consumption, which was 0.2-0.25 vvm. This value was very low in comparison with other studies, for example, in a case carried out in batch operation with ***Kluyveromyces fragilis*** in cheese whey, the aeration rate was 2 vvm. In this study, the yeast strain was cultivated in an open pond rectangular bioreactor at 30^0C, under non-sterile condition and uncontrolled pH. Initial COD of the medium culture was about 10000-20000 mg/l. The COD reduction attained 80% after 64 hours. The maximum suspended solids concentration obtained was 11.7g/l [11].

As mentioned above SVI is criteria for settle ability of activated sludge system. Figure 12 shows SVI values versus time for each HRT. As shown from this figure maximum value of SVI observed was 60 ml/g at HRT = 48 hours. Consequently, this yeast strain has high settling ability, so bulking was not occurred in this study. Normal range of SVI is 50-150 ml/g.

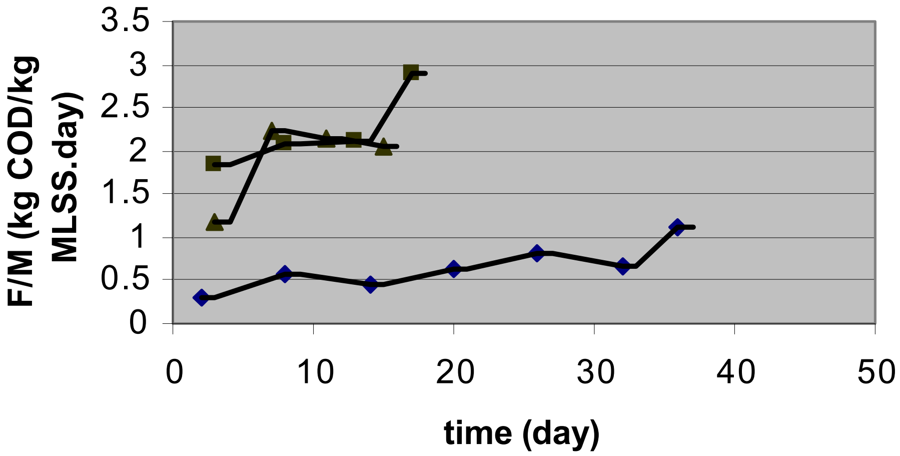


Figure 13. F/M ratios versus time for each HRT.♦: HRT= 48 hr; ■: HRT= 24 hr; ▲: HRT= 18 hr.

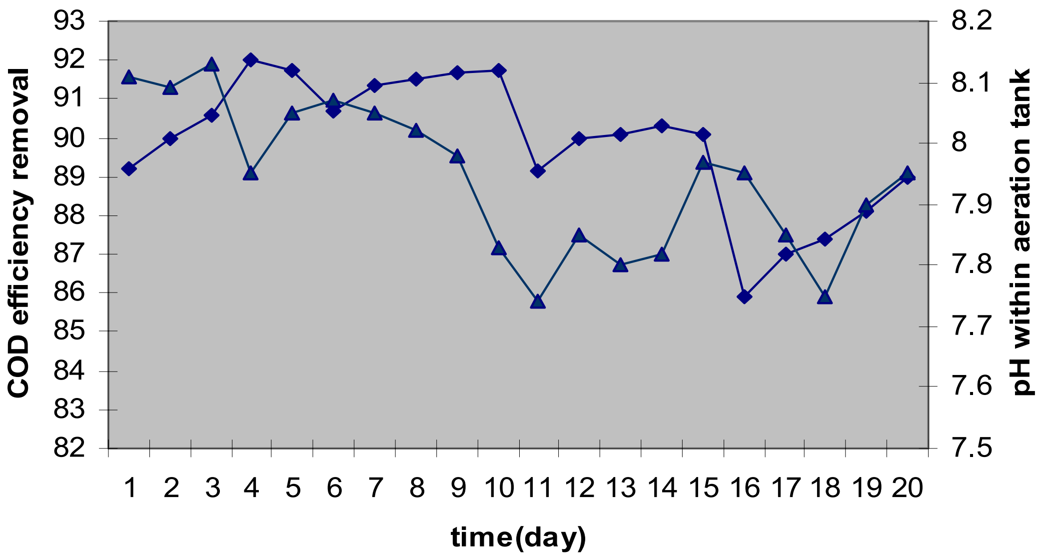


Figure 14. The pH values in the aeration tank versus COD removal efficiency for HRT= 24 hours. ▲: pH value; ■: COD removal efficiency.

For this system high F/M ratios were observed. Figure 13, shows F/M ratios versus time for each HRT. Maximum value of F/M ratio was 2.9 kg COD/kg MLSS. day, which was observed at HRT = 24 hours. For conventional aeration tanks, the F/M ratio is 0.2-0.5 kg BOD/kg. Day, but it can be higher for activated sludge using high-purity oxygen [6].

In this study, changes of pH within the aeration tank were monitored. The initial pH of feed (about 9) was dropped one unit approximately reaching to 8. Because, under aerobic oxidation, carbon dioxide and water are formed, and these two form carbonic acid in later stage. No relationships observed between COD, TOC removal with pH changes within the tank. This is shown at Figure 14 for HRT= 24 hours.

5. CONCLUSION

The yeast culture in the aeration tank promises a good potential replacement for conventional activated sludge. Because, sludge had low SVI, the yeast culture has a good settling.

Taking use of this system-bulking problem so can control ability. That is this yeast strain must be cultivated in the activated and by supplying required conditions (pH and temperature), which were different from normal conditions. So under such conditions the yeast strain was prevailed in activated sludge. On the other hand, this strain has shown high capacity to removal of COD and TOC.

Furthermore, the yeast has many advantages in comparison to other studies were carried out by various yeast strains, such as : required low aeration rate (0.2-0.25 vvm), using high organic loading rates and high F/M ratios and low hydraulic retention time (optimum HRT= 24 hours). Consequently, by using this system, bulking can be controlled with high performance.

6. NOMENCLATURE

K_d Endogenous oxidation rate or dead coefficient, day^{-1}

K_S Saturation coefficient, mg/l

S effluent dissolved COD, mg/l

S_0 Influent dissolved COD, mg/l

Y cell yield for COD, dimension- less

μ_m Maximum specific growth rate, 1/day

θ Hydraulic retention time, hour

X biomass concentration, mg/l

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