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Biological Treatment Processes: Suspended Growth vs. Attached Growth

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A B S T R A C T

Microorganisms play an important role in biological treatment processes. Biodegradable organic matter in wastewater would be removed completelyby proper microbial consortia. The mixed culture is either generally present in the wastewater, or is introduced to the target wastewater by mixing it with domestic wastewater or sewage sludge. However, pure cultures bioprocess may be used in certain cases for the removal of specific contaminants. In favorable environmental condition, microorganisms are capable of presenting high efficiency in destruction of pollutants. If environmental conditions such as nutrition, pH, temperature, dissolved oxygen, hydraulic and sludge retention time are suitable and consistent with the nature of process, microorganisms grow and remove organic pollutants through biochemical activities.The aim of this article was to investigate the performance and mechanism of various systems of suspended and attached growth; while the detail of process in different fabricated systems was evaluated. The advantages and disadvantages of NRBC, UASFF, UAPB and normal activated sludge process were discussed. Growth kinetics and COD removal of effluents were also investigated. The result indicated the combination of fluidized and fixed film has created active biogranules which were quite faster and more efficient in treatment of industrial wastewater.

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INTRODUCTION

Wastewater after primary treatments such as physical and chemical process may go through secondary treatment processes. The biodegradable soluble organic compounds are degraded through aerobic/anaerobic biological processes. Often, activated sludge process is well known among biological treatment. Beneficial microorganisms (bacteria and protozoa) feed on contaminants; their populations areincreasing (generating biomass). In aerobic oxidation process occurs while food and oxygen are supplied. In anaerobic organic matters are digested without presence of oxygen. In fact sludge is digested while organic compounds are hydrolyzed and volatile fatty acids are formed; then acid forms are taking action while methanogens are synthesizing methane and carbon dioxides[1]. Since the nature of organic compounds in wastewater in not defined; therefore, the term chemical oxygen demand (COD)and biochemical oxygen demand (BOD) are used to define the level of pollution. The term mixed liquor suspended and volatile suspend solids are also used in wastewater treatment. As the process generates sludge which indicates a high level of microbial activity and is used as a measurement of wastewater strength; as organic contaminants are removed. Once the organic compounds are utilized and converted to sludge the nature of treated waste is stable. The treated effluent goes to clarification, disinfection processes and then assurance of the healthy condition, the effluent is safe; has no significant organic pollution can expose to surface water reservoir for irrigation or other reuse[2, 3].

In suspended growth systems, such as activated sludge(also aerated lagoons and aerobic digestion) waste and microorganisms are combinedwhile oxygen, diffuse and penetrate into the cell. The free-floating microorganisms, gathering into biological flocs, settle out in clarifier. The settled flocs retained in a clarifier

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while part of the sludge is recycledto aeration tank. An proper ratio of recycled sludge would influence the performance of biological treatment [4].

Contrary to suspended solid systems, attached-growth processeshave support mediawhich they are fixed or moving.Microorganisms as a biofilm aremaintained and growthon the media and they get in contact with fresh wastewater.Trickling filters and rotating biological contactors (RBCs) are two popular attached growth processes which commonly are used in industrial wastewater treatment.The trickling filter,consists of a fixed bed media ofrocks, plastic material, or textile media.In this process wastewaterflows downward and passes and creates a biofilm on the media, that becomes thick and falls off when the thickness of biofilm increase considerably, this phenomenon is known as "sloughing" [5, 6]. Also, RBCs consist of a series of circular disks rotating through the wastewater flow, partially submerged. These rotating disks are usually plastic. Microorganisms as biofilm aredeveloped on exterior surface of the disks and eventually sloughs off if the film gets thick [7, 8].

The purpose of present work is to investigate the performance of various system of suspended and attached growth; while the detail of process in different fabricated systems were evaluated. The advantages and disadvantages of NRBC, UASFF, UAPB and normal activated sludge process were discussed.

MATERIALS AND METHODS

Analytical methods

In order to measure COD, a colorimetric method with closed reflux method was developed. Standard methods for examination of water and wastewater was used to measure COD and Lactose[9]. Spectrophotometer, UNICO 2100 (New Jersey, USA) at 600 nm was used to measure the absorbance of samples. Oxygen consumed is measured against standards at 600 nm with a spectrophotometer. A gas-tight syringe (Hamilton CO., Reno, Nevada, USA) was used to take sample from the gas sampling port. Gases chromatograph (Perkin Elmer, Auto system XL), equipped with thermal conductivity detector (TCD) and data acquisition system with computer software (Total Chrom), and were used for gas composition analysis. A GC column, Carboxen 1000, with 100/120 mesh (Supelco, Park, Bellefonte, PA, USA) was used. The column temperature was initially maintained at 40°C for 3.5 min, followed by automatic temperature increase at a rate of 20°C/min till it reached to 180°C. The injector and detector temperatures were 150 and 200°C, respectively. The carrier gas (He) flow rate was set at 30 ml/min. The concentration of carbohydrate solution was reacted with 3,5-dinitrosalycilic acid (DNS). The resultant change of color was linearly related to the carbohydrate concentration in the solution. The intensity of the color was recorded by using the absorbency test. A spectrophotometer at 540 nm wavelength was used. The cell optical density as biomass concentration was detected based on light absorbance and cell dry weights were measured. Since many bioreactors with different configurations are used; then, for the specific system definition and detail information is given in the related sections.The characteristics of the whey wastewater are presented in Table 1.

TABLE 1. Characteristics of waste stream

Parameter	Concentration (mg/l)
COD	60,000
BOD	32,500
TS	60,000
TSS	12,500
TKN	15
Lactose	57,500
pH	6.25
Alkalinity	003

RESULTS AND DISCUSSION

In biological treatment processes for domestic wastewater, two distinct methods are applied which known as aerobic and anaerobic processes. Activated sludge process is a very well defined aerobic process in domestic and industrial wastewaters. While for production of energy from domestic wastewater; anaerobic process is preferred. In old digestion tank, the process for production of hydrogen, methane and carbon dioxide required long retention time. The hydrolysis, acidifiers and methanogens are the stages required for the utilization of biodegradable organic materials in domestic wastewater. Long retention time of 24-28 days may require for the bioconversion of organic matters in domestic wastewater. Such process required to be modified and long retention time has to be reduced to 3-4 days [10, 11]. An up flow anaerobic fluidized bed biogranules along with fixed film (UAFF) bioreactors which are known as hybrid systems are employed to shorten the long retention time. In recent development, for the purpose of low power generation, microbial fuel cells (MFCs) are implemented for the bioconversion of organic maters in domestic wastewater. Our research team members are the front runner in MFCs; we published many papers and we are considering applying the biotechnology know how for commercialization of the biolamp [12].

Up-flow anaerobic sludge blanket (UASB)

The up-flow anaerobic sludge blanket reactor was developed in a few decades. In this process, wastewater is directly introduced to the bottom of the reactor, where it is uniformly distributed. The wastewater flows upward through a blanket of biogranules, which consume the waste as it passes through the blanket. Methane and carbon dioxide are collected by in the gas dome. Liquid passes through the bed where solid and liquid are separated. The solid retained in the blanket area while the liquid over flow from the weirs. Formation of granules and maintenance is extremely important in the process. In order to keep the blanket in suspension, an up flow velocity at 0.6-0.9 m/h was maintained. A full-scale plant for wastewater treatment of sugar beets industry achieved 80 percent removal of COD with organic loading rate of 10 kg COD/m3.d and HRT of 4 hours [13-15]. High rate systems like the up-flow anaerobic sludge blanket (UASB) and packed-bed reactors have been used for the treatment of various industrial wastewaters like dairy and brewery wastewaters [16-19].

Growth kinetics

Kinetic studies for the anaerobic digestion process of cheese whey were conducted in a pilot-scale upflow anaerobic packed bed bioreactor (UAPB). An influent COD concentration of 59419 mg/l was utilized at steady state condition. Logistic and Monod kinetic models were employed to describe microbial activities of Cheese whey in the anaerobic digester. The hydraulic retention times (HRT) in the range of 6 to 24 h were investigated throughout the experiment. Lactose conversions were 58.5 and 99.4 % for HRT of 6 and 16h, respectively. The methane productions rates were 6.57 and 3.25l/h for HRT of 6 and 24h, respectively. Biokinetic coefficients from Monod, K_{S}, μ_m and methane yield (Y_M) were 8.59, 7.63 (h^{-1}) and 0.11(g methane/g lactose), respectively. Table 2 summarized the kinetic data for several rate model applied for UAPB [13, 15, 20, 21].

Pilot scale UAPB bioreactor

Figure 1 presents the actual image of the pilot scale UAPB bioreactor. The system was packed with sea shells collected from Caspian Sea beach. The Plexiglas reactor was fabricated with an internal diameter of 19.4 cm and a height of 60 cm. The total volume of the reactor was 17.667 liters. The system was continuously operated without any disturbance for a period of 65 days [15, 21].

The UAPB was continuously operated with HRT of 6 to 24 h. The biofilm was fully developed on the natural packing (sea shell). Figure 2 shows substrate consumption profile (Lactose) of the effluents and the cell density with respect to HRT. The lactose concentration was sharply reduced to about 0.5 g/l at HRT of 16h. At any HRT of greater than 16h, the profile was flattened. The cell dry weight of the fermentation broth was analyzed at various HRT. As the HRT increased the cell density exponentially increased, at HRT of greater than 16h, the cell growth had reached to stationary phase [21, 22].

TABLE 2. Kinetic parameters, rate models with and without inhibition

Parameter	μ_m (h^{-1})	K_S (h^{-1})	ḱ (h^{-1})	K (h^{-1})	R^2	$Y_{CH4/lactose}$ (%)
Substrate utilization rate	-	-	0.175	-	0.99	-
Monod equation	7.633	8.6	-	-	0.99	-
Logistic equation	0.105	-	-	0.05	0.98	-
Methane yield	-	-	-	-	0.98	0.12

Figure 3 depicts methane production was gradually increased as HRT was step wised decreased (24, 20, 16, 13, 10, 9 and 6 h). As the flow rate was gradually increased the concentration of lactose also increased [21]. Figure 4 shows the SEM micrographs of the biofilm created by the anaerobic microbial consortia. The magnification scale is from 500 to 5000. The microbial core and brush shape are clearly shown. In these images, the support surfaces are fully covered by the active biofilm.

Up-flow anaerobic sludge fixed film (UASFF) bioreactor

The anaerobic sludge and fixed film bioreactor is a packed column, as anaerobic microorganisms grow on solid support, a film layer of attached growth of bacteria is developed on the packing media, a limited cell growth may take place. The filter may be operated up flow, which is also called up-flow fixed film reactor. The reactor can be operated down flow as well. Suitable packing such as raschig rings, flexirings, pall rings, rock or plastic balls and tubular raschig rings are required. Use of plastic balls size of 20 mm, resulted in a 40 % void volume. Also, PVC rings are often used. Keep in mind, the flow rate or up-flow velocity must be low about 1 m/h, without disturbing the biomass film and organic loading rate of 15 kg/ COD/m3/day is a desire flow rate [14]. The packed medium retaining biological solids and gas is produced in the digestion process. The fixed film portion positioned above the UASB section prevents sludge washout and helps to retain a high biomass concentration in the reactor. Several researchers have successfully used the UASFF reactor to treat various kinds of wastewaters such as starch, swine, slaughterhouse and antibiotic plant effluents [6, 7, 23-26]. An investigation on anaerobic digestion process of palm oil mill effluent (POME) was carried out in a pilot scale up-flow anaerobic sludge fixed film (UASFF) bioreactor at mesophilic temperature (38.8˚C). At steady state condition, the COD concentrations in feed stream were in the range of 5.26–34.725 g/l. The organic loading rates of POME were in the range of 0.88–34.73 g COD/l day. The hydraulic retention times (HRT) ranged between 1 and 6 days. Throughout the experiments, the removal efficiencies of COD were 80.6 and 98.6%. and methane production rate were 0.287 and 0.348 l CH_4 /g COD removed day [17, 27].

Figure 1. Actual image of UAPB bioreactor with working volume of 17.67L

Fluidized bed reactor

The fluidized or expanded bed reactor (FBR) is a new anaerobic treatment method.. In thiprocess, bacteria are grown on particles of medium such as sand or plastics which are absolutely fluidized and the wastewater flows upward through the bed. The effluent is recycled to mix with fresh feed in quantities by the wastewater and the fluidization velocity. The suspended biosolids have high rate of mass transfer and are biologically active. The particle size is about 0.7 mm. The advantage of fluidized bed reactor is that microbial over growth of biomass is absolutely reduced and SRT is much lower compare to other treatment system [16, 28-30]. FBR have been used for the treatment of phenolic wastewaters [29, 31]. Organic removal efficiencies of 80 percent were achieved at loadings rate of 4 kgCOD/m^3.d on dilute wastewaters. The range of acceptable organic loading, influent COD concentration is from 10 to 30 g/l. The loading rate of 1 to 30 kg COD/(m^3.d). The HRT is less than one day [32].

Activated sludge

A bench scale model was fabricated with working volume of 26 liters. The model was implemented for the domestic treatment of Yasreb-Ghaemshar's wastewater. Batch process of wastewater was used with additional nitrogen source for effective generation of sludge. Once sufficient amount of biomass was propagated carbon source from domestic wastewater is utilized. Fresh waste is transferred to the activated sludge unit. Wastewater was gradually added into about 10 liters of the suspension of biosolids. The effluents were collected, aged and settled in a settling basin then the sludge was returned to the aeration tank blended with fresh incoming wastewater. The recycle ratio of sludge was defined based on volume of return sludge to volume of fresh

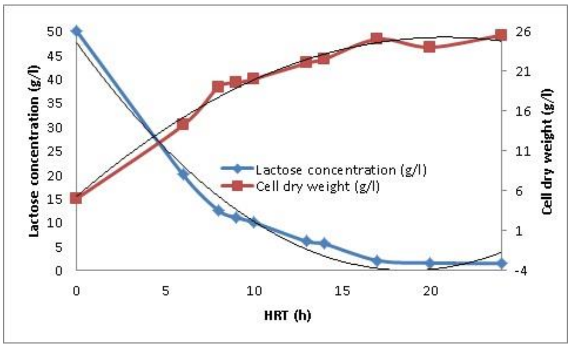

Figure 2. Lactose consumption profile and cell growth curve with respect to HRT

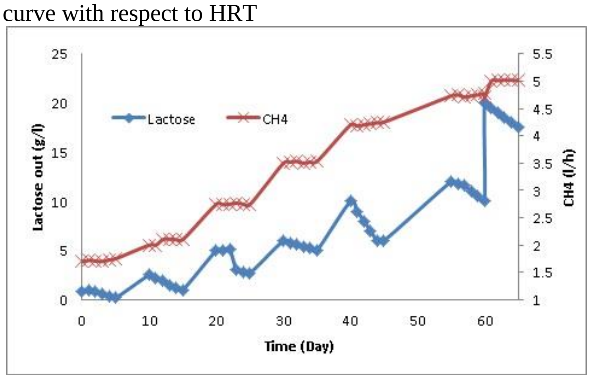

Figure 3. Methane production along with lactose concentration with stepwise reduction HRTs 24 to 6h

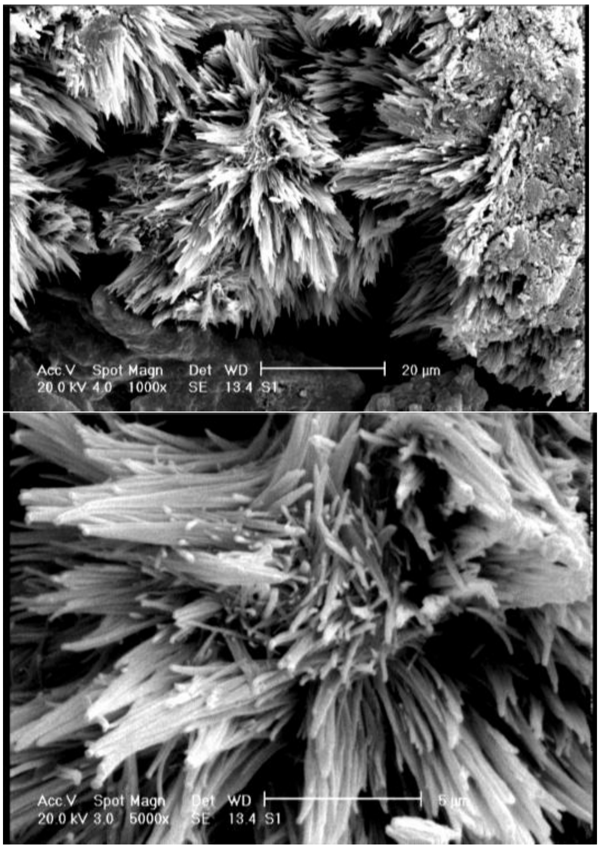

Figure 4. Biofilm of the microorganisms built on the surface of sea shells as packing

wastewater. The range of recycle ratio was from 2.5 to 40%. A 95% COD removal, as the maximum COD removal efficiency was obtained with 40% sludge retuned. The hydraulic retention time for the activated sludge process was set at 8hours. The sludge aged and the biosolids retention time was 23 days [33, 34]. The dissolved oxygen for 40% sludge return was stable at steady state condition measured at 3.5 mg/l. The pH was monitored and it was stable at 7.65. The growth kinetic model was obtained for the prediction and calculation of large scale model. The mass of sludge generated had a yield of ($Y_{X/S}$ = 0.53). The decay coefficient for the endogenous was reported. The experimental data obtained was quite promising and can be applied to the actual treatment plant.

Bulking sludge

In activated sludge system excess aeration may cause foaming and bulking which is floatation of low quality of sludge in an aeration tank. Once sludge is rising foaming and floating in the secondary clarifiers caused by poor settling characteristics and poor compactions, such phenomena is known as bulking sludge. Foam and poorly settling sludge are two of the most major problems of the activated sludge process. A sludge that exhibits poor settling characteristics is referred to as a bulking sludge. Filamentous microorganisms are usually responsibility for a bulked sludge. Large surface area to volume ratios for these microorganisms retards their settling velocities. Fungi are not normally significant in a wastewater treatment. Fungi are the most familiar filamentous microorganisms. The vegetative structure of the most fungi is composed of filaments. Nutritional characteristics of filamentous sulfur bacteria (*Thiotrix*, *Beggiatoa*, and *Leucothrix*) have been investigated. *NocardiaMicrothrixparvicella* are associated with extensive foaming in activated sludge processes. The foam originated by*Nocardia*is thick and brown color. Spraying chlorine on the surface of clarifier may eliminate *Nocardia*. It was found that at low F/M and low DO < 0.5 mg/l, filamentous organism population increases, causes bulking. Prechlorinated wastewater may prevent the growth of filamentous bacteria.

Activated sludge kinetics

Theoretical aspect of activated sludge mostly discussed about food to microorganism ratio (F/M), sludge retention time (θ_C) and COD removal. The data obtained were fitted by the kinetic models described in this paper and also the kinetic coefficients were determined. The F/M was defined as:

$$\frac{F}{M} = \frac{S_O}{(\tau)(X)} \qquad (1)$$

where, S_O is influent substrate concentration (mg/l), τ is hydraulic retention time (day) and X is the biomass concentration (mg/l). The specific rate of substrateutilization (U) is defined as the product of F/M and treatment efficiency (E).The relation present U is obtained by substitution of equation 1 for the F/M and yield resulted as:

$$U = (\frac{F}{M})E = \frac{S_O - S}{(\tau)(X)} \qquad (2)$$

The specific rate of utilization can be measured by substrate utilization per unit mass of organisms $U = -r_S / X$. The rate of substrate utilization is defined by Monod equation [2, 17, 19, 35, 36]:

$$-r_S = \frac{\mu_m XS}{Y(K_S + S)} \qquad (3)$$

In equation 3, the term μ_m / Y is often replaced by a constant k, defined as the maximum specific substrate utilization rate, then equation 5 is simplified for rate model:

$$U = \frac{kS}{K_S + S} \qquad (4)$$

The mean cell residence time in the activated sludge system (θ_C) is measured by as stated in the following equation:

$$\frac{1}{\theta_C} = YU - k_d \qquad (5)$$

The COD removal and effluent COD with respect to sludge age is depicted in Figure 5.

Based on equation 4, Figure 6shows the double reciprocating utilization rate and leaving substrate; the data are well fitted with the projected model. Based on equation 5; the rate of utilization of organic matters is strictly related to sludge retention time which is the age of active sludge (see Figure 7).

A combined systems of NRBC and UASFF

The cheese whey used in as a source of high strength wastewater was obtained from Gela Factory, Amol, Iran. Based on experimental demands, various dilutions of wastewater were prepared using distilled water. pH of the wastewater was maintained at 6.5 nearly neutral conditions by addition of 1M NaOH. The image of RBC constructed in lab and biofilm developed from the treatment of cheese processing effluents on disks are shown in Figures 8 and 9, respectively.

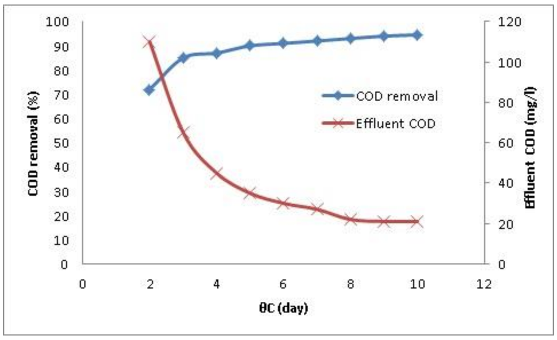

Figure 5. COD removal and effluent COD with respect to sludge retention time

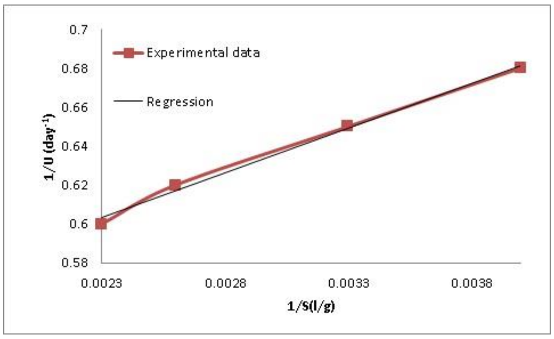

Figure 6. Growth kinetic for activated sludge double reciprocating utilization rate and leaving substrate

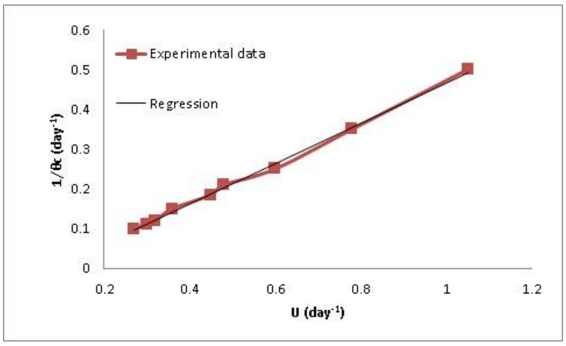

Figure 7. Sludge age relation with utilization of organic matter in the wastewater

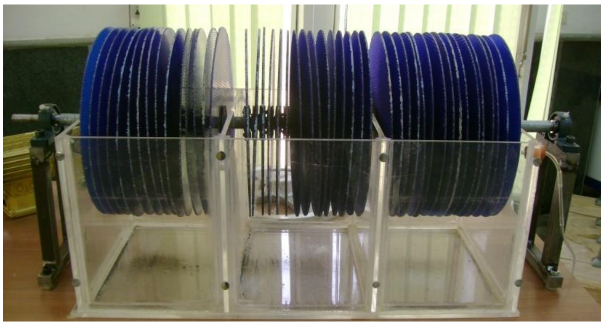

Figure 8. The image of fabricated NRBC

Figure 9. Biofilm developed on rotating discs of the NRBC system

A pilot scale three-stage RBC with nominal volume 80 liters connected to an up flow anaerobic sludge fixed film bioreactor (USAFF). The UASFF bioreactor was followed by NRBC system. The effluent stream of the NRBC was introduced into the UASFF for further treatment. The schematic diagram of NRBC and UASFF bioreactor is shown in Figure 10.

After development of a permanent and uniform biofilm on both sides of the discs, the system was operated in full capacity. In order to investigate the effects of influent COD and HRT on COD removal efficiency, various influent COD (40,000, 50,000, 60,000, 70,000 g/l) and HRT (8, 12, 16 h) were selected. The system was operated in sufficient time at each selected HRT to establish the steady condition. The effect of HRT on COD removal efficiency is depicted in Figure 11. It was observed that for all influent COD concentrations, the COD removal efficiency increased as HRT was gradually increased from 8 to 16 h. Maximum COD removal efficiency of 82% was obtained at HRT of 16 h and influent COD of 50,000 mg/l. As the influent COD was increased to 60,000 and 70,000 mg/l, COD removal efficiency was gradually decreased that was due to the high organic load shocks.

Figure 12(a), (b) and (c) refer to the wastewater samples which were pretreated at HRT of 8, 12 and 16 h in the NRBC system, respectively. The achieved data revealed that the COD removal efficiency increased as the HRT was gradually increased. Figure 12(a) shows that maximum total COD removal efficiencies of 95.7, 96.9, 96.6 and 97.1% at total HRT of 24 h (8 h in NRBC and 16 h in UASFF) were obtained for the influent COD of 40,000, 50,000, 60,000 and 70,000 mg/l, respectively. Figure 12(b) and (c) depict, similar trends were followed for samples which were pretreated for 12 and 16 h in the NRBC reactor.

Anaerobic mechanisms

Organic matters presenting as both electron donor and electron acceptor cause this phase to be referred to as

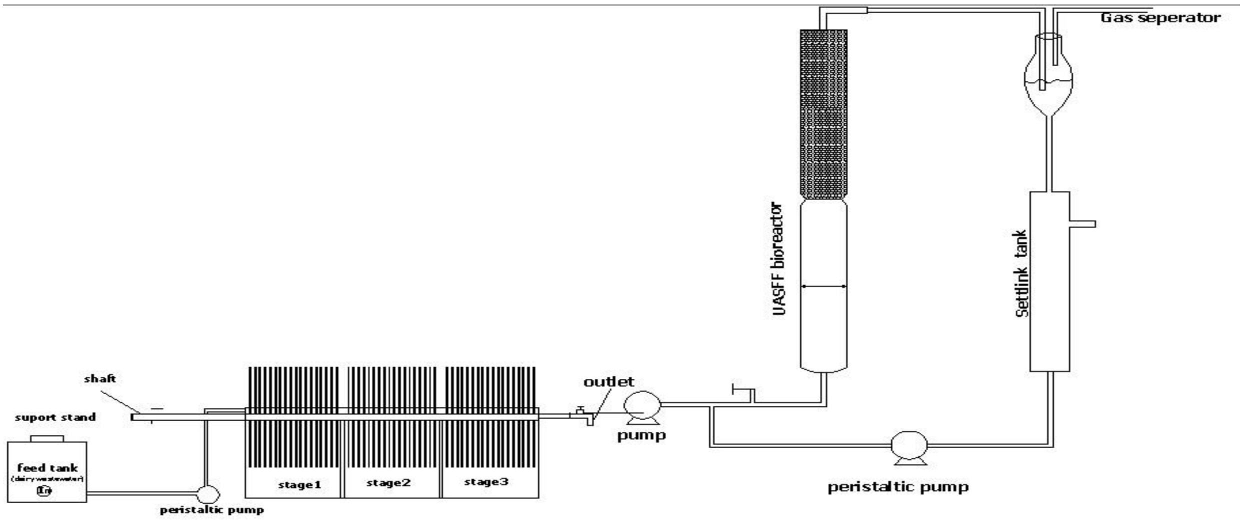

Figure 10. Schematic representation of the UASFF bioreactor fed with effluent of the NRBC system

fermentation phase. It is also called as acidogenesis because production of simple organic acids occurs in this phase. Depending on the nature of organic waste, anaerobic process may initiate from either hydrolysis or fermentation. Bacteria that perform hydrolysis and fermentation are of both facultative and obligate anaerobic groups. For instance, fermentation is the initial step for some industrial wastewaters. In the last stage, known as methanogensis, a number of microorganisms are involved which contribute to methane formation. Methanogens can be categorized into three groups: aceticlastic methanogens, hydrogen-utilizing methanogens, and acetogens that each group is in charge of specific reactions. As acetate is cleaved by aceticlastic methanogens, methane and CO_2 is formed from its methyl group and carboxyl group, respectively. Hydrogen-utilizing methane-formers oxidize hydrogen while CO_2 is reduced so as methane is produced. The third group, namely acetogens, uses CO_2 as electron acceptor and H_2 as electron donor to form acetate, which is further converted to methane. Methane-former organisms, classified as archaea, are of strict obligate anaerobes [12]. Figure 13 illustrates the summary of stages and phases as complex organic matters are reduced to methane and CO_2. Development of sulfate-reducing bacteria in anaerobic processes can be troublesome as sulfide which is toxic to methanogens is produced. These organisms can reduce sulfate to sulfide, while they are classified into two groups considering their electron donors [37**,** 38]. One group is capable of oxidizing a wide variety of organic materials to acetate when sulfate is reduced to sulfide. Second group consists of organisms which can consume fatty acids, especially acetate, in order for producing CO_2 alongside reducing sulfate to sulfide. To prevent sulfide toxicity, controlled addition of iron instance in the form of ferrous or ferric chloride to the wastewaters containing sulfate is recommended since sulfate and iron react, thus, precipitate as iron sulfate.

CONCLUSION

It was concluded that each process may have advantages or disadvantages. In comparison in terms of biomass generation aerated system behave as a fast treatment system while anaerobic processes are considered as a slow process. The modern and advanced hybrid processed deal with biogranules formation may be much faster than conventional anaerobic processes. Mass of sludge generation in aerated units are may be 10 times high than the anaerobic processes. Based on characteristic of wastewater a specific combined process may be recommended.

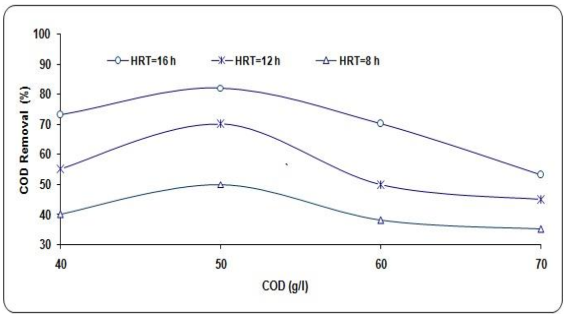

Figure 11. Effect of HRT on COD removal efficiency in the RBC system

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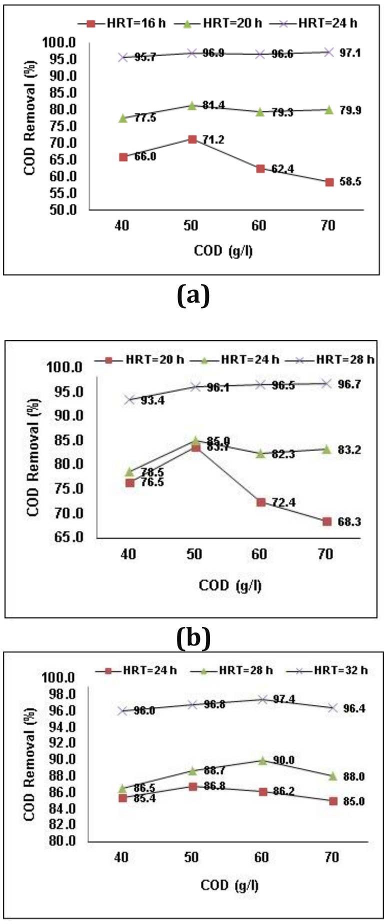

(a) **(b)** **(c)**

Figure 12. COD removal efficiency with respect to HRT in the UASFF bioreactor (a) pretreated samples at HRT= 8 h, (b) pretreated samples at HRT= 12 h, (c) pretreated samples at HRT= 16 h

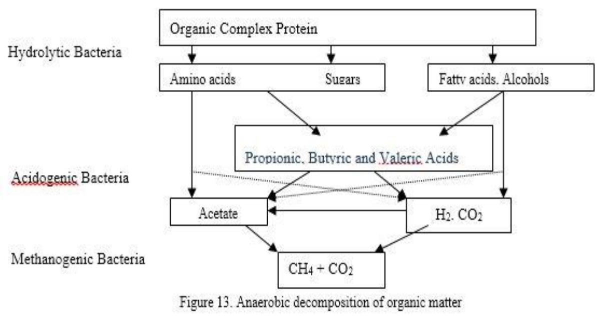

Figure 13. Anaerobic decomposition of organic matter

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Persian Abstract

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چکیده

با توجه به نقش مهم میکرواورگانیزم ها در فرآیندهای تصفیه بیولوژیکی فاضلاب، مواد آلی قابل تجزیه توسط مخلوط مناسبی از میکرواورگانیزم ها قابل حذف می‌باشد. به طور کلی محیط کشت مخلوط همواره در فاضلاب وجود دارد و یا از طریق مخلوط کردن فاضلاب مورد نظر با بخشی از فاضلاب شهری یا لجن فاضلاب بدست می‌آید. از طرف دیگر استفاده از کشت خالص میکرواورگانیزم ها برای حذف آلاینده‌های خاص مناسب می‌باشند. به همبن دلیلی زمانی که شرایط محیطی، از قبیل مواد غذایی، pH، دما، اکسیژن محلول، سن لجن و زمان ماند هیدرولیکی مطلوب باشند میکرواورگانیزم ها رشد کرده و قادر به حذف آلاینده‌های آلی از طریق فعالیت‌های بیوشیمیایی خواهند بود. هدف از این مقاله تحقیق در مورد عملکرد سیستم های مختلف میکرواورگانیزم ها شامل فرآیندهای رشد چسبیده و رشد معلق است. همچنین جزییات فرآیند در سیستم های ساخته‌شده مختلف توصیف شده است. مزایا و معایب سیستم هایی از قبیل NRBC، UASFF، UAPB و لجن فعال متعارف ارزیابی گردیده است. سینتیک‌های رشد و اکسیژن خواهی شیمیایی پساب خروجی از سیستم های مختلف مورد بررسی قرار گرفته است. نتایج نشان داده است ترکیب فرآیندهای بستر ثابت و بستر معلق سبب پدید آمدن گرانول های بیولوژیکی فعالی شده که در تصفیه فاضلاب‌های صنعتی بسیار کارآمد می‌باشند..