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Comparison of Adenosine Triphosphate and Oxygen Uptake Rate As Biological Process Parameters

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ABSTRACT. Adenosine triphosphate (ATP), a biochemical compound detected only in living cells, and oxygen uptake rate (OUR) were used to characterize the viability and metabolic activity of activated sludge microorganisms in batch and continuous flow systems operated at three sludge ages: $\theta c = 2.5$ days; $\theta c = 5$ days; and $\theta c = 10$ days. OUR responded rapidly to substrate addition to acclimated and starved microorganisms and indicated the cell's metabolic activity, while ATP remained relatively unchanged and reflected the concentration of viable biomass. A maximum viability of 100 percent was defined at the minimum sludge age (θ_C^M) estimated to be approximately 1.9 days. The viability of activated sludge solids decreased as the sludge age increased and was estimated to be, relative to the assumed viability at the minimum sludge age: 75 percent at $\theta c = 2.5$ days; 45 percent at $\theta c = 5$ days; and 25 percent at $\theta c = 10$ days. ATP and OUR, combined with total suspended solids (TSS) and volatile suspended solids (VSS), should enhance the operation and control of activated sludge systems.

The increasing emphasis on water quality control and the inherent inadequacies of conventional parameters motivated investigators to look for new parameters that reflect the viability of microorganisms. Viability determines the effectiveness of biological treatment systems. The concentration of microorganisms, as measured by total suspended solids (TSS) and volatile suspended solids (VSS), does not necessarily reflect viability. The simplicity and reliability of the TSS and VSS

measurements favoured their continued use. Nevertheless, researchers continue to investigate new parameters to reflect the quantity of viable biomass. Christensen and McCarty (1975) divided the VSS of activated sludge into viable and inactive biomass, with the viable biomass fraction estimated to be in the range 10 percent to 50 percent (Weddle and Jenkins 1971, Patterson *et al.* 1970, McKinney 1962).

Several parameters have been used to characterize the viability of microorganisms. In the direct microscopic count method, it is difficult to distinguish between living and dead cells. In the plate count method, it is difficult to find a growth medium to support all microorganisms. Biochemical parameters such as protein, organic nitrogen and deoxyribonucleic acid (DNA) are limited by the fact that nonviable solids contain such substances. Adenosine triphosphate (ATP) measurement, on the other hand, have the following characteristics: 1-all living cells contain ATP; 2-no ATP is found in non-living matter; 3-ATP disappears rapidly from the cell after it dies; 4-ATP can be measured easily and rapidly; and 5-ATP level in the biomass can reflect environmental stresses such as the level of dissolved oxygen and toxicity. The level of adenosine triphosphate (ATP) in activated sludge microorganisms was estimated to be in the range 0.1 to 2.2 mg ATP per g VSS (Patterson *et al.* 1970, Weddle and Jenkins 1971, Williamson and Nelson 1981). Roe and Bhagat (1982) estimated the ATP content of a completely viable sludge to be 5.51 mg/g TSS. The oxygen uptake rate (OUR) measurement reflects both, the concentration of viable microorganisms and the level of metabolic activity. Edwards and Sherrard (1982) observed that the specific OUR (OUR/VSS) ratio fluctuated depending on the influent organic loading. Benefield *et al.* (1979) estimated the viable fraction of activated sludge using the specific OUR measurement.

While the OUR measurement is well established and widely used in practice, the use of ATP measurement remains a research tool. The accumulation and confirmation of knowledge regarding the use of ATP remain important. Both parameters, ATP and OUR, indicate the viability of activated sludge solids. However, little data is available for useful comparison of the two parameters. The objectives of this study was to evaluate and compare ATP and OUR measurements as biological process parameters. Batch and continuous flow experiments were conducted to provide the data necessary for the evaluation.

Materials and Methods

Three laboratory-scale, activated sludge reactors were operated at sludge ages (θc) of 2.5 days, 5 days, and 10 days. Each 8-liter reactor was divided into two basins: an aeration basin (5.5 liters) and a clarifier (2.5 liters). Each reactor was

operated for a total period of three times the sludge age to ensure the achievement of steady-state conditions. The excess sludge was removed daily from the aeration basins. The synthetic feed was pumped at one-liter per hour. The synthetic substrate (Table 1) was prepared after Hartz *et al.* (1985). Air was supplied to each reactor through diffuser stones.

Table 1. Synthetic substrate composition

Component	Quantity	
Bacto-pepton	350	mg/L
Yeast extrac	20	mg/L
Urea	325	mg/L
NH_4Cl	21.3	mg/L
$FeSO_4 . 7H_2O$	2.8	mg/L
$MnSO_4 . H_2O$	5.1	mg/L
K_2HPO_4	697	mg/L
KH_2PO_4	272	mg/L
Influent pH	7.6-7.8	
COD	450	mg/L

For immediate oxygen uptake measurement, samples were transferred from the reactors into 300 mL BOD bottles and the dissolved oxygen (DO) readings were taken every 30-seconds for a 10-minute period. The dissolved oxygen readings were plotted versus time and the oxygen uptake rate was determined as the slope of the line of best fit. The influent and effluent chemical oxygen demand (COD), and the TSS and VSS in the effluent and aeration basins were measured every day. ATP and OUR were also measured in the aeration basin every day. All analytical procedures, including DO, TSS, VSS, and COD were measured using standard procedures (APHA 1989).

The batch-scale experiments were conducted in a two chamber, four-liter per chamber respirometer. OUR was continuously recorded during the batch experiments. The substrate was added to the reactor while the microorganisms were in the endogenous respiration phase (respiration in the absence of external food or respiration of starved microorganisms). The procedure for adding the substrate included withdrawing an activated sludge sample from the reactor, settling the withdrawn sample, wasting a volume of the supernatant equal to the volume of the substrate sample, and replacing the sample containing the substrate inside the reactor. The substrate sample consisted of diluted wastewater with a COD of 88 mg/L.

ATP measurement was based on the luminescence reaction in fireflies (McElroy 1947). The firefly extract contains luciferane and the enzyme luciferase. The reaction leading to light emission in fireflies can be simplified as follows: luciferane + luciferase enzyme + ATP = light energy + other products. Seliger and McElroy (1960) stated that one quantum of light is emitted for each molecule of ATP that is hydrolyzed and that the light produced is directly proportional to the amount of ATP present in the sample.

Verstrate *et al.* (1983) provided a detailed discussion of the use of nucleotide releasing reagent for Bacteria (NRB) to extract ATP from soil microorganisms. In this study, NRB was used for ATP extraction from activated sludge microorganisms. Detailed procedures, and quality assurance and control checks were reported elsewhere (Shanableh 1988). The instrument used for ATP analysis was a 3M company Lumac/3M biocounter (Model M2010A). The biocounter is capable of producing a linear light output response in the range of 0.2-pg to 1-ng (pg = picogram = 10^{-12} g; ng = nanogram = 10^{-9} g) ATP per 100-µL sample (Lumac/3M 1985). All samples were diluted to produce a light output response within the linear range of the instrument. The dilution solution, Tris-EDTA-NaN_3 (TEA buffer) was prepared after Verstrate *et al.* (1983). One-mL activated sludge sample was transferred into a flask that contained the dilution solution. Diluted samples were frozen at –22°C, when necessary, for later ATP analysis. The quantity of ATP per gram TSS (Figs. 1 and 3) was estimated using ATP calibration standards. Accordingly, the reported ATP/TSS quantities should be interpreted with caution because the ATP standards did not contain the same interferences as in the activated sludge samples.

Results and Discussion

In aerobic metabolism, ATP is produced mainly in the electron transport-chain by a process called oxidative-phosphorylation. In the process, electrons from the substrate molecules are passed to the final electron acceptor, oxygen, by a set of cyclic intermediate electron acceptors. In oxidative-phosphorylation, a direct relationship between oxygen consumption and ATP generation can be established. In uninhibited biological environments, the relationship between OUR and the rate of ATP generation, $R_{ATP,generation}$, can be expressed by the relationship presented in Equation 1.

$$OUR = K_{ATP} * R_{ATP,generation} \quad \ldots\ldots (1)$$

where K_{ATP} is a constant. The ATP produced in the cell is continuously consumed to

maintain life activities. Brock and Madigan (1988) estimated that during the time the cell doubles, the cell's ATP content is consumed and regenerated about 10,000 times. Accordingly, a mass balance on the ATP in the cell can be expressed as follows: net rate of ATP change = ATP generation rate – ATP consumption rate. The mass balance can be translated into the following differential equation:

$$R_{ATP,net} = R_{ATP,generation,} - R_{ATP,consumption} \quad \ldots\ldots (2)$$

The batch experimental results presented in Figures 1(a) and 1(b) indicate that the metabolic activity of the starved biomass responded rapidly to the addition of substrate as manifested by the rapid and significant increase in OUR. For example, the data presented in Figure 1(a) show that OUR increased from approximately 110 (mg O_2 per g TSS per day) to 550 (mg O_2 per g TSS per day) immediately after the addition of the substrate. The quantity of ATP increased gradually [Figure 1(a)] from approximately 200 μg ATP per g TSS to 240 μg ATP per g TSS (approximately 20 percent increase) reflecting the increase in the metabolic activity of the microoganisms. Little biomass growth was observed as the TSS concentration remained within the range 530 mg/L to 550 mg/L during the experiment. The initial increase in ATP was followed by a gradual decrease reflecting the declining metabolic activity after the external substrate was consumed.

Similar observations were made when three increasing quantities of the substrate were added, Figure 1(b), sequentially. OUR responded rapidly and significantly to the addition of substrate while ATP remained relatively unchanged after each addition. The overall concentration of ATP decreased gradually with time reflecting the aging of the biomass and the insufficient food quantities for growth and maintenance. No significant growth was observed as the TSS remained approximately 540 mg/L during the experiment.

To compare the response of both parameters, the relative variations (percent change) of the ATP and OUR data in Figure 1 are shown in Figure 2. The data in Figure 2 indicate that OUR was a better indicator of the metabolic activity of the biomass than ATP. The response of OUR to substrate addition was immediate and significant, increasing in the range 400 percent to 500 percent. The ATP response was gradual and small compared with the OUR response. The response of OUR to substrate addition implied that the metabolic activity of the biomass achieved a maximum value and that a minimum reaction time was required to utilize the substrate. The difference in the response of ATP and OUR to substrate addition can be explained using Equations 1 and 2. The addition of substrate (food) resulted in an immediate increase in the metabolic activity of the starved microorganisms and a

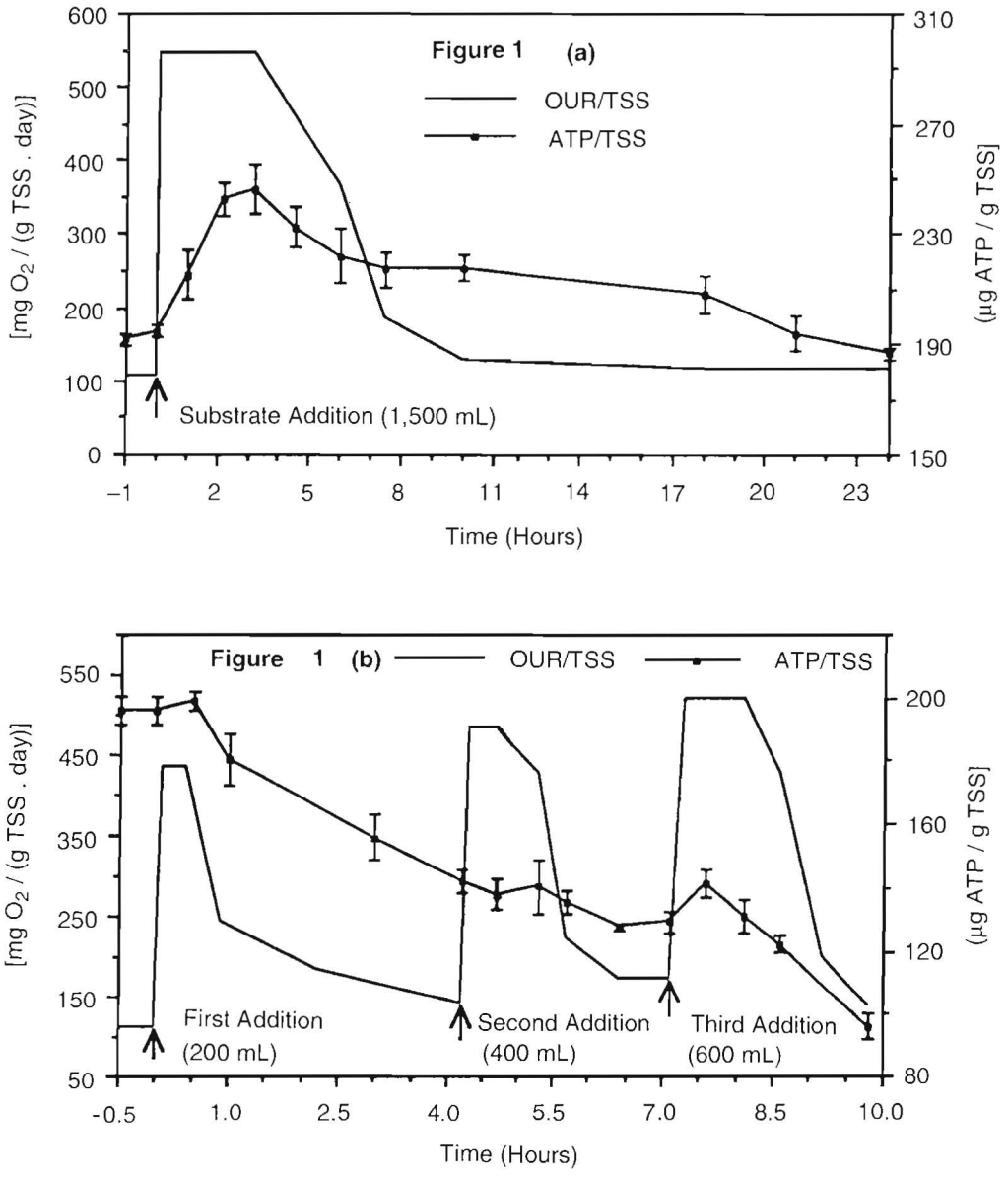

Fig. 1. Response of ATP and OUR to substrate addition.

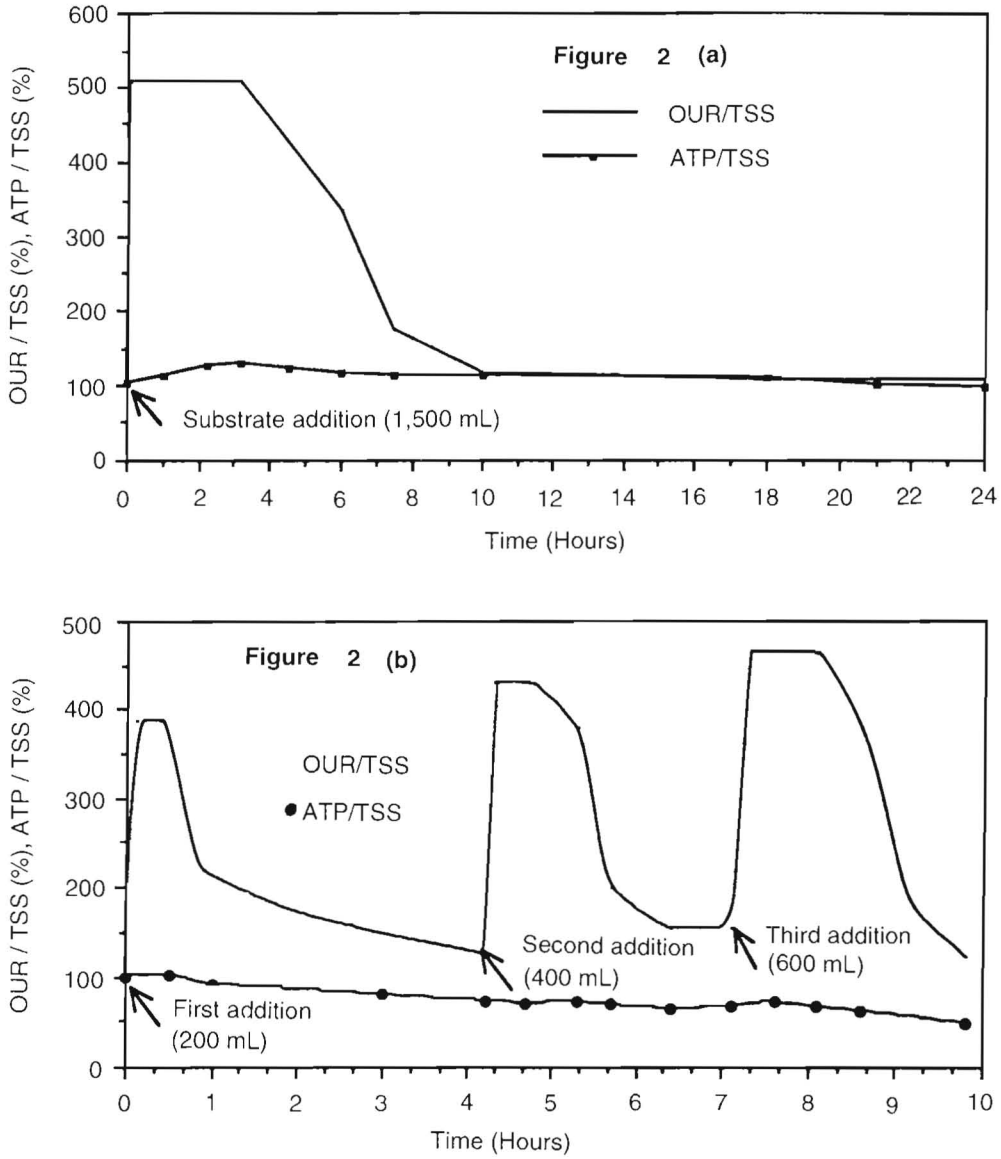

Fig. 2. Relative response of ATP and OUR to substrate addition.

rapid oxygen uptake. According to Equation 1, the increase in OUR was accompanied by a similar increase in ATP generation rate. A similar increase in ATP consumption rate (Equation 2) prevented accumulation of ATP in the cell. The increase in ATP generation rate ($R_{ATP.generation}$) was partially balanced by an increase in ATP consumption rate ($R_{ATP.consumption}$) resulting in the small and gradual ATP increase observed in Figure 1(a). ATP provided a reasonable estimate of the quantity of viable biomass in the system under varying substrate loading conditions, while under the same conditions, OUR indicated the level metabolic activity. OUR may reflect the amount of viable biomass provided that the substrate-biomass relationship is defined and is kept constant.

The data presented in Figures 3(a), 3(b), and 3(c) indicate that the specific ATP (ATP/TSS) in the continuous flow activated sludge reactors generally increased during the transient-state (after startup) then stabilized as the systems approached steady-state conditions. During the transient state, the nonviable biomass was continuously replaced by viable biomass through the cycles of biomass growth and wasting. In addition, the environmental conditions continued to improve as the biomass became acclimated to the new substrate in an oxygen rich environment. Also during the transient-state, the microbial population acclimated and shifted from one that favoured domestic waste to a population adjusted to the new substrate and environment. The specific oxygen uptake rate (OUR/TSS) showed little variations during the transient period and achieved a relatively constant value at steady-state. Both ATP and OUR reflected the viability of the biomass under steady-state conditions as the substrate to biomass ratio was defined and maintained constant.

Because of continued recycling, nonviable solids accumulate in activated sludge systems operated at high sludge ages thus reducing the viable fraction of the solids. On the other hand, activated sludge systems can not be operated below a minimum sludge age (θ_C^M). The sludge age is maintained through sludge wasting and recycling. Without recycling, the system can not retain microorganisms as those will be washed out by the wastewater flow. With some recycling, the sludge age is maintained above zero. A minimum sludge age is required to prevent the microorganisms from being washed out of the system. Accordingly, a sludge age below the minimum value is not defined and the maximum viability and activity of the biomass in the system occur when the sludge age (θ_C) corresponds to its minimum defined value, θ_C^M. The minimum sludge age is achieved, theoretically, when waste stabilization does not occur. θ_C^M is defined as in Equation 3 (Metcalf and Eddy 1985).

$$\frac{1}{\theta_C^M} = \frac{YkS_o}{K_s + S_o} - k_d \quad \text{..... (3)}$$

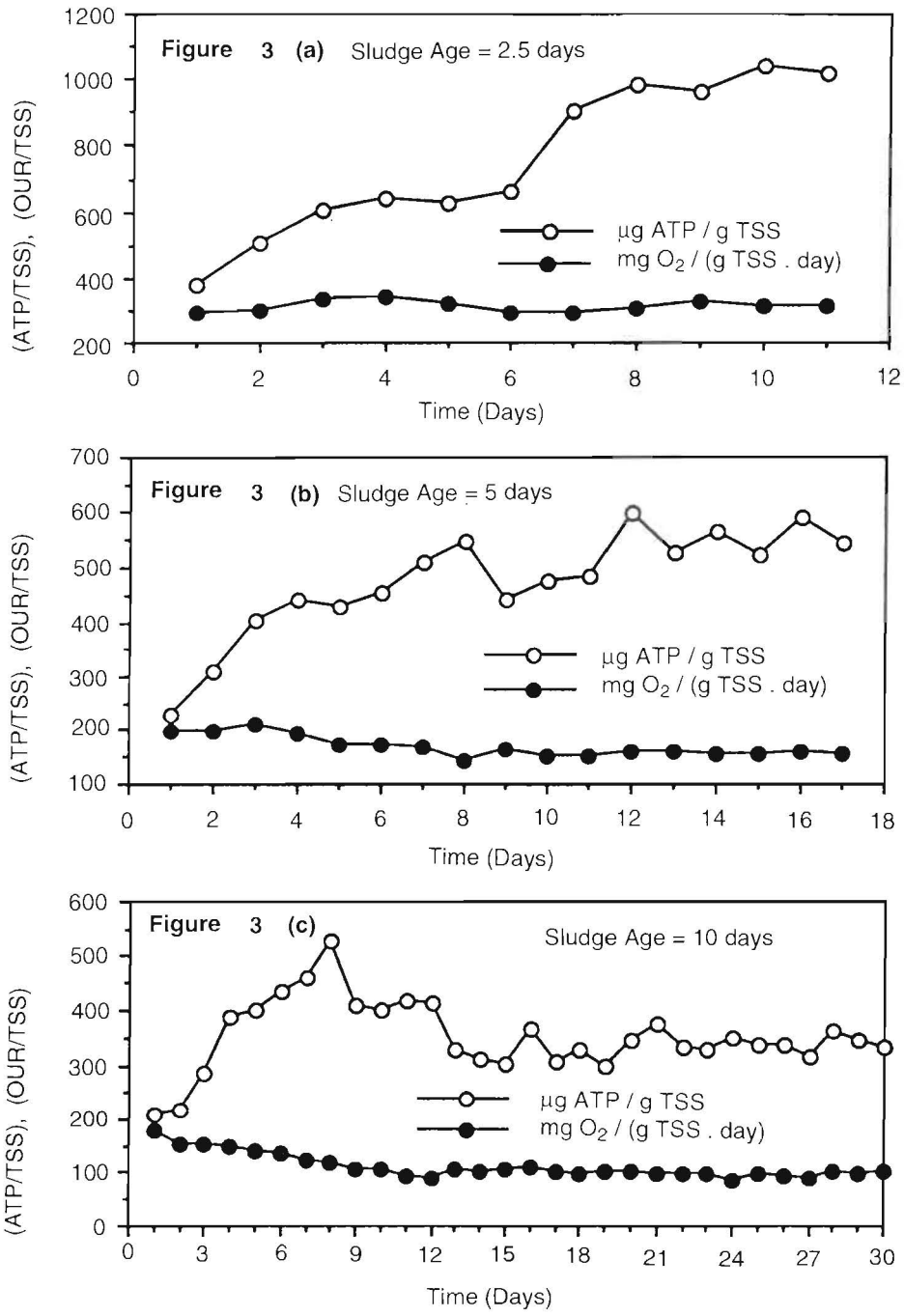

Fig. 3. Variations of activated sludge viability during transient and steady states.

where Y = yield coefficient (mass/mass), k = constant ($time^{-1}$), K_S = concentration constant (mass/unit volume,) So = influent COD concentration (mass/unit volume), and kd = decay coefficient ($time^{-1}$). Using the steady-state data presented in Figure 3, the variations of ATP/TSS with θc were calculated and are presented in Figure 4. The maximum ATP/TSS and OUR/TSS values were determined by extrapolating the curves back to the minimum sludge age (θ_C^M) calculated (Shanableh 1988), based on Y = 0.77 mg TSS/mg COD, k_d = 0.1 day^{-1}, k = 1 day^{-1} and K_S = 110 mg/L COD, to be approximately 1.9 days. To compare the viability of activated sludge solids relative to their vibility at the minimum sludge age, it was assumed that the viability at the minimum sludge age was equal to 100 percent. Based on this assumption, the relative viabilities were approximately (Figure 4): 75 percent at θ_C = 2.5 days, 40 percent at θ_C = 5 days and 25 percent at θ_C = 10 days. The average steady-state TSS concentration was 1540 mg/L in the reactor with θ_C = 2.5 days, 2810 in the reactor with θ_C = 5 days, and 4270 mg/L in the reactor with θ_C = 10 days. Accordingly, the viable TSS concentration in the three reactors at steady-state, relative to the maximum value, was estimated to be 1155 mg/L in the reactor with θ_C = 2.5 days, 1130 in the reactor with θ_C = 5 days, and 1070 mg/L in the reactor with θ_C = 10 days. The trend indicates that the viability level in the three reactors was approximately similar. However, the available experimental data is not enough to support such a conclusion and further research is required to investigate the above observation.

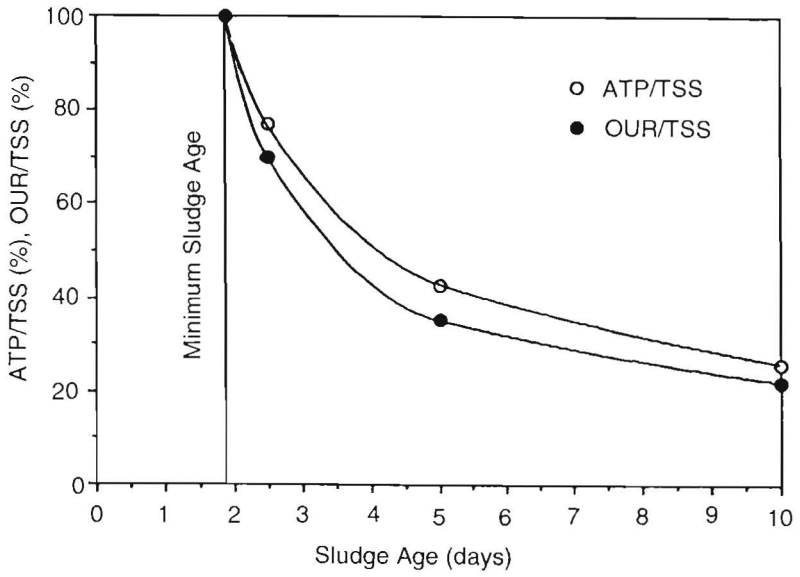

Fig. 4. Relative viability of activated sludge solids.

Conclusions

(1) OUR responded rapidly and significantly to substrate addition to starved and acclimated microorganisms and indicated the cell's metabolic activity.

(2) As the metabolics activity increased, the rates of ATP generation and consumption increased. The change in ATP level in the biomass is determined by the difference between the rates of ATP synthesis and consumption. ATP indicated the amount of viable biomass under varying substrate availability conditions.

(3) OUR can be used to estimate the viable biomass if the substrate-biomass relation is well defined and is kept constant.

(4) Activated sludge microorganisms achieve maximum viability and activity when $\theta_C = \theta_C^M$. The viability and activity of activated sludge microorganisms increase rapidly as θ_C approaches θ_C^M, and reach their maximum values at $\theta_C = \theta_C^M$.

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مقارنة الأدينوسين ثلاثي الفوسفات وسرعة إستهلاك الأكسجين كمعاملات بيولوجية

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ان وجود مركب الأدينوسين ثلاثي الفوسفات في خلايا الكائنات الحية وعدم احتواء المواد الغير حية على هذا المركب يجعله مناسباً كمقياس لحيوية الكائنات الدقيقة في الحمأة المنشطة . ولذلك ، فقد استخدم هذا المركب ، بالاضافة إلى سرعة استهلاك الأكسجين ، لقياس حيوية الكائنات الدقيقة في الحمأة المنشطة تحت ظروف متغيرة من حيث توفر الغذاء الخارجي وكذلك في مراحل نمو مختلفة . وقد استخدمت مفاعلات هوائية تحتوي على كائنات دقيقة متوسط أعمارها يومان ونصف ، وخمسة أيام ، وعشرة أيام . وقد دلت التجارب على أن سرعة استهلاك الأكسجين استجابت بسرعة وازدادت بنسفة وصلت إلى ٥٠٠ بالمئة عندما أضيفت المواد الغذائية للكائنات التي حرمت من الغذاء سابقاً ، بينما ازدادت كمية الأدينوسين تدريجياً بنسبة لا تزيد على العشرين بالمئة لتعكس نمو الكائنات الدقيقة وازدياد نشاطها . وقد استنتج انه يمكن استخدام مركب الأدينوسين لتقدير نسبة الكائنات الحية في الحمأة المنشطة تحت ظروف تغير كمية الغذاء بينما يمكن استخدام سرعة استهلاك الأكسجين لتقدير سرعة النمو وسرعة استهلاك الغذاء الخارجي .