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Microbial Kinetics and Growth Study in Biological Digestion of Composite Tan Liquor

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Abstract: In the present paper, description of anaerobic treatment of tannery wastewater, according to the proposed kinetic model, has been presented. The experimental results of microbial growth predictions showed the greatest resemblance to the conventional models. The study on cell growth kinetics, substrate uptake and product formation in microbial growth, and enzyme kinetics has been carried out. The studies showed that an optimum BOD influent load of 0.8 kg BOD/m^3/day with three days retention time could be adopted to yield about 97 percent BOD reduction. The bio-kinetic coefficients were evaluated using modified Monod's equations to study the metabolic performance of the digestion process. The role of magnesium carbonate during anaerobic digestion has been studied for the enhancement of methane generation.

Key words: Anaerobic digestion, bio-kinetics, substrate growth, Monod's equation.

Introduction

Environmental pollution has become major concern in developing countries in the last few decades. Major sources of water pollution are the untreated or partially treated industrial effluents. Tanning industry is reputed globally as major industry which contributes to water pollution. The quality of discharged water from tanneries is far from the desired level of acceptance into water ways.

A tannery discharges from 21,500-21,950 litres a day, corresponding to 86-88 litres per kg of leather processed. Chromium is known to be highly toxic to the living aquatic organism in the hexavalent state and somewhat less toxic in the trivalent form. The effluents from chrome tanning industry shall meet the specific tolerance limits for chloride with 1000 mg/L, BOD (5 day at 20°C) with 30 mg/L, hexavalent chromium with 0.1 mg/L and pH between 5.5 and 9.0.

Tanning of animal hides to convert them into leather is an important industrial activity. But the pollution from tanneries has a long-term negative impact on the environmental resources. The liquid waste from tanneries is a dangerous pollutant because it contains organic matter and inorganic pollutants in the solution, in suspension as well as in colloidal dispersion. Hence, there is a need to remove these pollutants before they are released to render them harmless. In the past ten years, a number of different anaerobic processes have been developed for the treatment of industrial wastes

Anaerobic digestion is one of the oldest processes used for the stabilization of sludges. It involves the decomposition of organic and inorganic matter in the absence of molecular oxygen. In this process, the organic matter in the mixture of primary settled and biological sludges is converted biologically, under anaerobic conditions, to a variety of end products including methane and carbondioxide. The process is carried out in an airtight reactor. Sludge, introduced continuously or intermittently, is retained in the reactor for varying periods of time. The stabilized sludge, withdrawn continuously

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or intermittently from the reactor, is reduced in organic and pathogen content and is non-putrescible.

Kinetics

Biochemical Reaction

Food + O_2 + nutriennts $\xrightarrow{\text{Bacteria}}$ CO_2 + NH_3 new biomass + other end products.

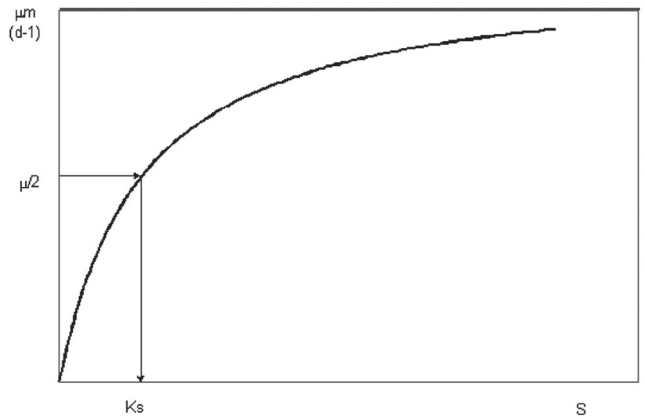
Biomass Concentration

The concentration of biomass, X (mg/L), increases as a function of time due to conversion of food to biomass:

$$\frac{dX}{dt} = \mu X$$

where μ is the specific growth rate constant (d^{-1}). This represents the mass of cells produced/mass of cells per unit of time.

Effect of substrate concentration on growth rate constant



Growth Rate

Growth rate constant, μ, is a function of the substrate concentration, S. Two constants are used to describe the growth rate.

μ_m (mg/L) is the maximum growth rate constant (the rate at which the substrate concentration is not limiting).

K_s is the half-saturation constant (d^{-1}) (i.e., concentration of S when μm = $\mu_m/2$

$$\mu = \mu_m \frac{S}{K_s + S}$$

Biomass Production

$$\frac{dX}{dt} = \text{Growth rate} - \text{Death rate} = \mu X - k_d X$$

where k_d represents the endogenous decay rate (d^{-1}) (i.e., microorganism death rate).

Substituting the growth rate constant:

$$\frac{dX}{dt} = \left(\frac{\mu_m S}{K_s + S}\right) X - k_d X$$

Substrate Utilization

$$\frac{dS}{dt} = \frac{1}{Y}\frac{dX}{dt}$$

where Y is the yield factor (mg of biomass produced/mg of food consumed)

$$\frac{dS}{dt} = \frac{1}{Y}\frac{dX_{\text{growth}}}{dt} = \frac{1}{Y}\left(\frac{\mu_m S X}{K_s + S}\right)$$

Food to Microorganism Ratio (F/M)

It represents the daily mass of food supplied to the microbial biomass, X, in the mixed liquor suspended solids, MLSS. Units are kg BOD_5/kg MLSS/day.

$$\frac{F}{M} = \frac{BOD_5(kg/m^3) \times \text{Influent flow}(m^3/d)}{\text{Reactor Solids}(kg/m^3) \times \text{Reactor Volume}(m^3)}$$

$$= \frac{S_0 Q_0}{XV}$$

Since the hydraulic retention time, $\theta = V/Q_o$, then

$$\frac{F}{M} = \frac{S_0}{dV}$$

Literature Review

Studies on the properties of chromium sludge from chrome tan liquor and related sludge volume, sludge settling rate, surface loading rate etc. have been reported (Pathe et al., 1995). The treatment of tannery and electroplating effluents by using lime, NaOH and their mixture in the temperature range of 25 to 100°C was investigated (Shukla and Shukla, 1994). The study on a laboratory scale completely mixed continuous flow activated sludge system to treat settled chrome tannery wastewater was carried out and observed the BOD and COD removal ranged from 84 to 96% (Gurusamy et al., 1995). The experiments on the activated sludge treatment of vegetable tanning waste admixtured with 10%, 25%, and 50% settled sanitary sewage showed a BOD removal from 87 to 96% (Elangovan et al., 1995).

It was reported that the pH of unpolluted soil was high, whereas that of polluted soil was low. The electrical conductivity and chloride content were high and it was unfit for the growth of plants. The heavy metals in all the samples were adequate for the growth of plants

(Mariappan et al., 2001). Alkalotolerant/alkalophilic actinomycetes NCIM 5080 and NCIM 5142 produce alkaline protease in presence of chromium ions. Both the actinomycetes are able to grow in undiluted tannery effluents and remove chromium almost completely and reduce the COD by 70%-80% during growth as well as by pregrown biomass (Snehal et al., 2001).

A laboratory scale experiment was conducted on aerobic digestion of tannery effluent using cowdung as the seed material. The BOD removal of 95.8 per cent was obtained at an optimum organic load of 0.6 kg BOD/m^3/d. Biokinetic coefficients were calculated for the data obtained to study the metabolic performance of the microorganisms (Prakash, 2001). Adsorption technique has been applied for the removal of hexavalent chromium from aqueous solution using wheat straw dust, saw dust, and coconut jute, and the results are compared with the powdered activated carbon. The high uptake of hexavalent chromium was observed with PAC at pH 2.0, and for the other adsorbents at pH 6.0 (Rao and Bhola, 2000). An attempt has been made to assess vermiculite, a phyllosilicate mineral group with high cation exchange capacity, as an alternative for activated carbon (Jayabalakrishnan and Mahimai Raja, 2007).

The design of any biological wastewater treatment system must depend on the proper relationships between the organic matter in the wastes and the microorganisms which can metabolize the organic matter, the generation time of the microorganisms, the temperature of the treatment system, pH, the nutrient elements in the waste, the wastewater retention period in the system and other environmental factors. Bio-kinetics is based on the actual environment and the biological metabolic activities in the system. Hence, the design of biological wastewater treatment based on biokinetics will have a better control over environment and biological community in the system. For a specific waste, a biological community and proper set of environmental conditions, the biokinetic coefficients are fixed. Hence, design of biological treatment system based on the bio-kinetic parameters will be more rational than many of the modern designs.

Several quantitative mathematical models have been developed over the period to describe the kinetics of tannery waste treatment processes. However, successful application of these models to design is contingent on the use of a number of kinetic parameters which, in turn, depend on the nature of the wastewater. The values of biokinetic parameters for tannery wastewaters are not widely available for the biological treatment systems. Hence there is a need to evaluate these parameters for anaerobic systems. To accomplish this objective, experiments were conducted using chrome tanning wastewaters for the treatment.

A comprehensive review of the methods for handling tannery effluent showed that the effluents from such plants are generally high in both dissolved organic and inorganic materials, posing particular treatment difficulties. Although a number of treatment procedures are being used or have been proposed, there is no widespread agreement on the most suitable method. Also information on the design of treatment plants based on biokinetic parameters for tannery effluent is very limited, the prime objective of the present study is to determine the biokinetic parameters which enable us to describe the metabolic performance of the microorganisms when fed with the substrate and other components in the tannery treatment processes.

Experiment

The experiment was designed and operated on the principle of an anaerobic activated sludge process to evaluate the bio-kinetic parameters, which could be used in the rational design and operation of large-scale anaerobic installations. The reactor was a wide mouthed Pyrex glass bottle of five litre capacity as shown in Figure 1. The reactor has provision for adding wastes, for removing treated effluent and settled solids and for gas transfer. The gas collection apparatus consisted of a glass bottle of two litre capacity and another bottle of one litre capacity for the water displaced from the gas bottle. Care was taken to remove the air from the reactor as well as from the gas collection bottle at the beginning of the experiment and the entire set up was checked for gas leaks. Tubes were connected to the digester to facilitate feeding of the waste and removal of the effluent. The digester was kept in water bath at a constant temperature of 35°C. Cow dung was used as the seed material and fed into the digester to start with. After establishing necessary biota from cow dung sludge, the chromium-free composite liquor is fed into the digester daily. The pH of the influent sample was adjusted to pH 7 by adding alkali before feeding. After feeding, the contents in the digester were given thorough mixing by manual shaking. The BOD load was kept at 0.25 kg BOD/m^3/day in the beginning. After several displacements of the digester contents and after establishing stable conditions of digestion the loading rate was gradually increased. Gas measurements were done once a day. The gas was burnt periodically to confirm the presence of methane which formed a major portion of a gas.

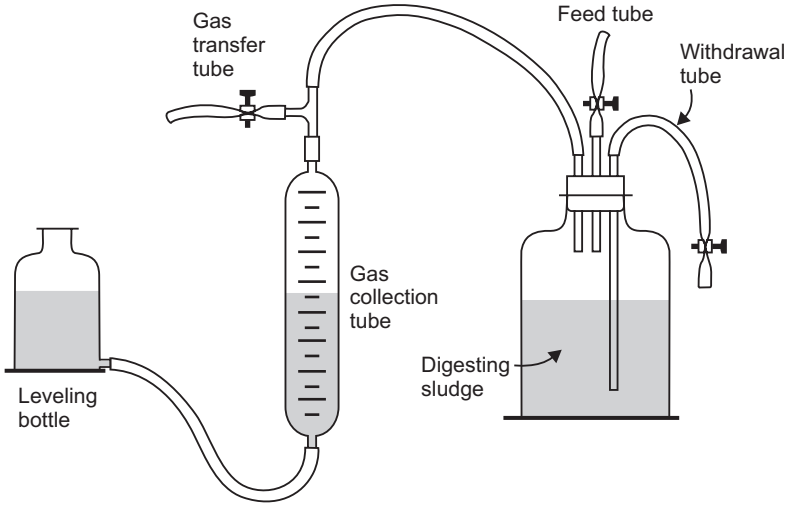


Figure 1: Anaerobic reactor used for the experiment.

The samples of effluents drawn at various stages were analyzed for pH, influent BOD (So), effluent BOD (Se), mixed liquor volatile suspended solids (MLVSS) before sludge wasting, initial MLVSS and the net growth rate of microorganisms $\Delta X/\Delta t$ which was obtained from the difference of MLVSS before sludge wasting and initial MLVSS values. The pH was maintained within the optimum range of 6.8 to 7.4 which is favourable for anaerobic bacterial growth. Calculated amount of diammonium phosphate and urea were added to the feed solution as and when required in order to maintain the BOD : N : P ratio at 100 : 2.5 : 0.5 which is effective for anaerobic digestion. In anaerobic digestion, biomass is formed having a molecular formula $C_5H_7O_2N$. Cell synthesis requires nitrogen (amino acid formation) for which nitrogen (in the form of urea) rich nutrient is supplied.

During cell synthesis, energy in the form of ATP is released for which phosphorus acts sink. The contents in the reactor were continuously mixed with the help of magnetic stirrer. The tannery wastewater was filled up to a volume of two litres in the anaerobic reactor and the mixture was mixed daily at frequent intervals. Neither waste feeding nor withdrawal of mixed liquor was done until gas production was noticed. Regular wasting and feeding were continued until a steady state condition was reached. The daily BOD loading rate was kept constant at around 0.3 kg/m^3/day. The daily gas production, the influent and effluent BOD, Mixed Liquor Volatile Suspended Solids (MLVSS) which indicates the concentration of microorganisms in the reactor, pH, volatile acids and alkalinity were recorded at the steady state condition at which the sludge growth and gas production remained constant. The mean cell residence time was varied by operating the reactor at several MLVSS concentrations.

Results and Discussion

The general characteristic properties of composite tannery effluent are presented in Table 1. The result indicates that the liquor is basic with pH 7.8. Optimization of pH for chrome reduction is shown in Figure 2. The chromium content has been found to be 120 mg/L and the BOD and COD of the effluent have been estimated to be 1360 and 2510 mg/L respectively. The results indicate that the effluent has to be treated for an effective removal of chromium before being subjected to biological treatment. Lime was used as the precipitating agent for chromium removal and effect of lime on chrome precipitation is presented in Table 2. It was observed that the chromium

Table 1: General characteristics of composite tan liquor

Parameter	*Value*
pH	7..8
Alkalinity (as $CaCO_3$)	1100
Total solids	22400
Total dissolved solids	20890
Total suspended solids	1510
Volatile suspended solids	810
Chlorides	7600
Sulphates	2840
BOD	1360
COD	2510
Chromium	120
Sulphide	90

All values except pH are expressed in mg/L.

Table 2: Effect of lime on chromium precipitation

Weight of lime added (g/L)	*pH*	*Chromium in filtrate (mg/L)*
2.0	8.1	3.64
2.5	8.3	2.89
3.0	8.5	2.66
3.5	8.6	2.00
4.0	8.8	1.64
4.2	8.8	0.90
4.4	8.9	0.40
4.6	9.1	0.54
5.0	9.4	0.66
5.5	9.9	0.68

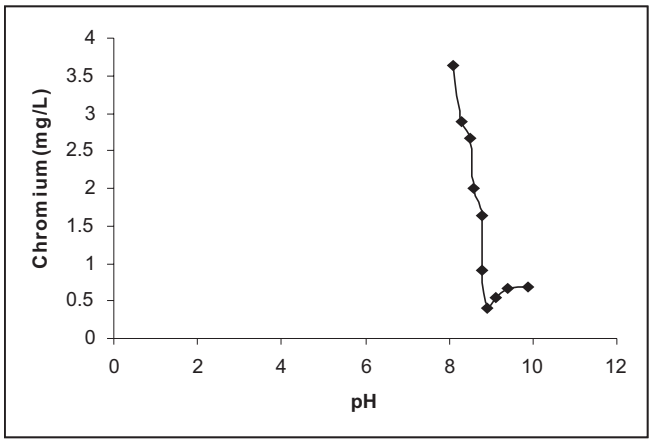


Figure 2: Optimization of pH for chrome reduction.

removal increased with increase in pH and the maximum chromium removal of 99.7% was observed at pH at 8.9 with a lime dose of 4.4 g/L. Further increase in lime has resulted in the decrease of chromium removal due to redissolution of the mixture under such experimental conditions. The results of the anaerobic digestion of the chrome-free composite liquor are presented in Table 3. The data consists of varying BOD loading rate changes in pH alkalinity, volatile acid, and percentage BOD reduction. It was observed that a maximum BOD reduction of 96.9% was obtained at the BOD loading rate of 0.80 kg BOD/m^3/day and throughout different loading rates, the BOD reduction was more than 94% which could be due to the proper maintenance of alkalinity and volatile acids in the digester. In the beginning of the process, the pH of the effluent was 6.9. As the loading increased gradually the pH increased to 7.6 up to the optimum loading and dropped down slightly to 7.4 at the maximum loading. The increase in alkalinity was steady as the loading increased gradually. Side by side there was a production of volatile acids but was not considerable. With the initial pH correction and with proper seeding of the waste, the process of digestion was taking place unhindered, without undue accumulation of intermediate products. There was no possibility for the formation of free volatile acids. BOD Removal Efficiency with varying organic load is represented in Figure 3.

Table 3: Anaerobic digestion of composite liquor

BOD load (kg BOD/m^3/ day)	*pH*	*Alkalinity (as $CaCO_3$) mg/L*	*Volatile acids (mg/L)*	*% BOD reduction*
0.25	6.9	340	40	94.6
0.30	7.0	410	60	95.0
0.35	7.2	560	76	95.2
0.40	7.4	950	110	95.4
0.50	7.4	980	144	96.0
0.60	7.6	1460	168	96.2
0.70	7.6	1880	190	96.8
0.80	7.6	1920	236	96.9

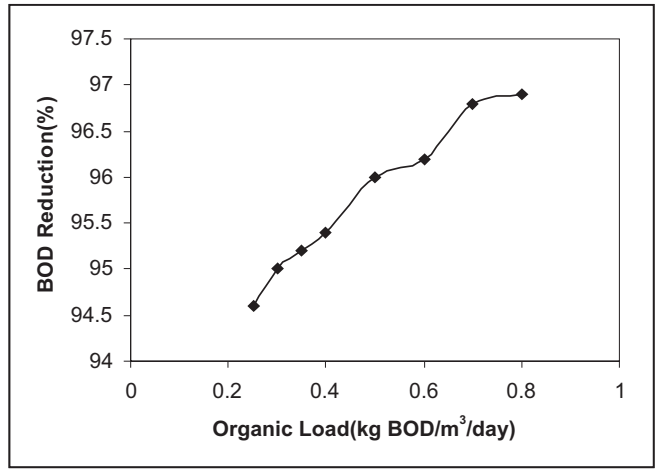


Figure 3: BOD removal efficiency with load.

Due to initial pH correction the alkalinity level in the waste was boosted up and this gradually increased at every increase in loading. This helped in maintaining adequate buffer capacity in the digester to neutralize the volatile acid. Much of the alkalinity to build up the digester may also be due to the release of ammonia from nitrogenous organic matter in the waste undergoing digestion. The volatile acids also react with the alkalinity formed and form an acid salt with release of carbon dioxide. The salt in turn reacts with acid and appears as part of the alkalinity. Methane organisms are extremely sensitive to pH values. They are most reactive in the pH range 6.6 to 7.2. In the present study, the influent pH was adjusted to 7.0 using magnesium carbonate which,

apart from raising the pH is also useful for methane organisms as a source supply of CO_2 in producing additional quantities of methane. The reaction may be represented as follows:

$$MgCO_3 + 2H_2O \rightleftharpoons Mg(OH)_2 + H_2CO_3$$

$$H_2CO_3 \longrightarrow H_2O + CO_2$$

This is a reversible reaction. Initially there was certain amount of $Mg(OH)_2$ produced which helped to neutralize the acidity in the raw waste. It was possible that during anaerobic digestion of the waste, the methane organisms might utilize the CO_2 gradually for forming methane since CO_2 is a hydrogen acceptor.

$$4\ H_3A + CO_2 \longrightarrow 4\ A + CH_4 + 2\ H_2O$$

where A is any oxidized substrate.

The presence of traces of H_2S in the gas may be due to the reduction of sulphates present in the waste. Although the presence of higher sulphide concentrations affect volatile acid production and methane fermentation during an aerobic digestion, the presence of less amount of volatile acids in the experiment seemed to indicate that methane fermentation has in no way been affected. The variation of alkalinity with organic load is shown in Figure 4. The volatile acid production was kept under control and this may be due to the high level of alkalinity maintained in the digester. The optimum and the maximum values of the treated liquor is shown in Table 4. The biokinetic coefficients were evaluated using modified Monods equations and are represented in Table 5. The rate of substrate utilization was found to be higher at the early stages of digestion throughout the processes and the reason for the initial high substrate utilization rate may be due to the adsorption of soluble substrates by the bacteria and extra cellular slime. The subsequent drop in rate following the initial high rate may be interpreted as saturation of adsorption sites. The subsequent rate increase may be attributed to continued increase in metabolic activities caused by cell growth. Adsorption and metabolism occur concurrently. The BOD reduction with time is shown in Figures 5 and 6.

Table 4: Results of the treated liquor

Parameter	*Maximum*	*Optimum*
BOD load, kg BOD/m^3/day	0.80	0.60
pH	7.6	7.4
BOD reduction (%)	96.9	95.4
Volatile matter reduced (%)	80	76
Alkalinity (as $CaCO_3$) mg/L	1920	980
Volatile acids (mg/L)	236	168
Methane production (%)	34%	28%

Table 5: Bio-kinetic coefficients

Parameter	*Value*
Substrate removal rate constant, k/day	1.66
Half velocity constant, Ks, mg/L	1132
Decay constant, Kd, day^{-1}	0.05
Yield coefficient, Y	0.22
Maximum specific growth rate of Microorganisms, μ_m/day	0.368

The kinetic rate constants for the anaerobic digestion of tannery effluent at an optimum organic load of 0.8 kg BOD/m^3/day are represented in Table 6. It was observed that the concentration of the reactant decreases and that of the product increases exponentially with time. The constancy values of rate constants confirm a first order reaction.

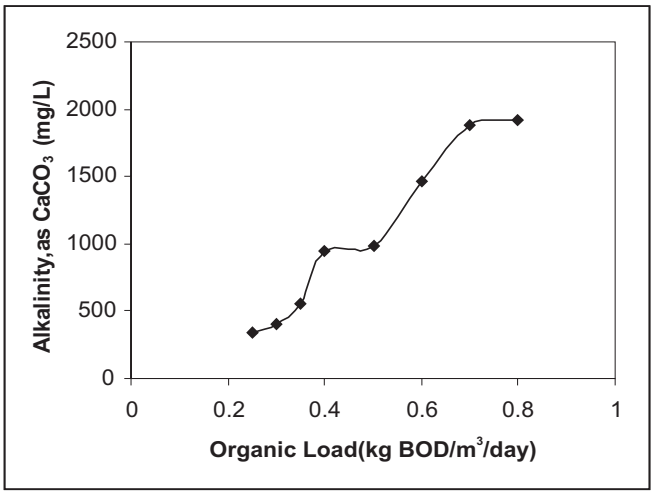


Figure 4: Alkalinity variation with load.

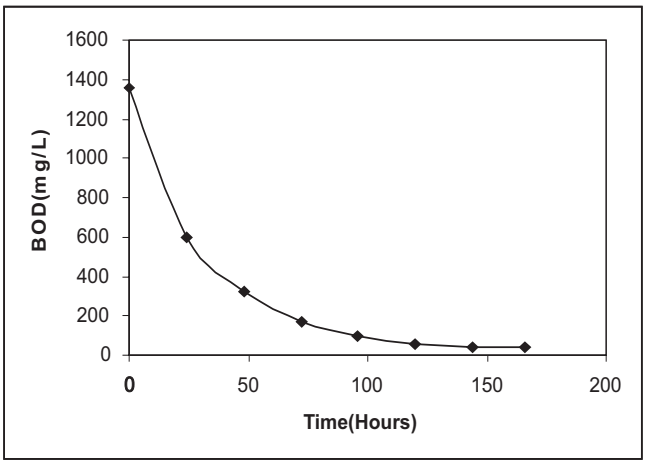


Figure 5: BOD reduction with time.

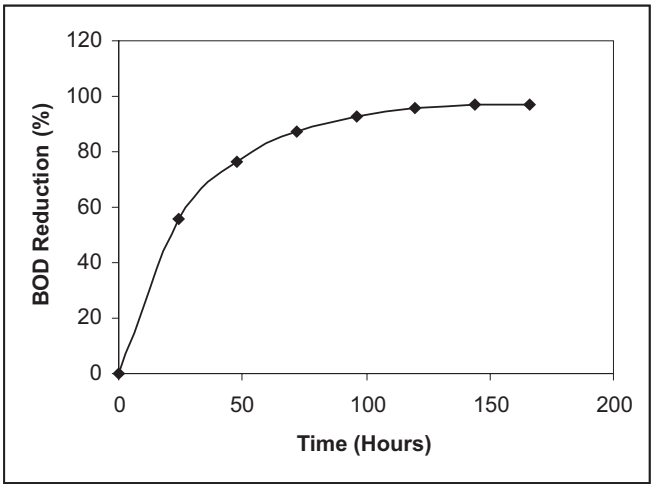


Figure 6: % BOD reduction.

Table 6: First order rate constant values

Time (hours)	*Rate constant, k*
24	0.0301
48	0.0208
72	0.0204
96	0.0253
120	0.0260
144	0.0245

Microbial growth can be defined as an orderly increase in cellular components, resulting in cell enlargement and eventually leading to cell division. This definition is not strictly accurate as it implies that a consequence of growth is always an increase in cell numbers. However, under certain conditions growth can occur without cell division, for example, when cells are synthesizing storage compounds, e.g. glycogen or poly-b-hydroxybutyrate. In this situation the cell numbers remain constant, but the concentration of biomass continues to increase. This is also true for coenocytic organisms, such as some fungi, that are not divided into separate cells. Their growth results only in increased size.

Figure 7 shows the variation of substrate concentration with time. The growth modification is due to the bacterial binary fission in homogeneous suspension cultures, where cell division produces identical daughter cells. Each time a cell divides is called a generation and the time taken for the cell to divide is referred to as the generation time. Therefore, the generation time or doubling time *(td)* is the time required for a microbial population to double. Theoretically, after one generation, both the microbial cell population and biomass concentration have doubled. However, as previously stated, under certain conditions growth can be associated with an increase in biomass and not cell numbers. The biomass concentration during the digestion process is represented in Figure 8. When a graph is plotted of cell biomass against time, the product is a curve with a constantly increasing slope. During exponential growth, when all nutrients are supplied in excess and are therefore non-limiting, there is a direct relationship between cell numbers and biomass concentration, assuming that mean cell size is constant.

Cell division occurs with increasing frequency until the maximum growth rate (mmax) for the specific conditions of the batch fermentation is reached. At this point exponential growth begins and cell numbers/ biomass increase at a constant rate. Mathematically, this exponential growth can be described by two methods;

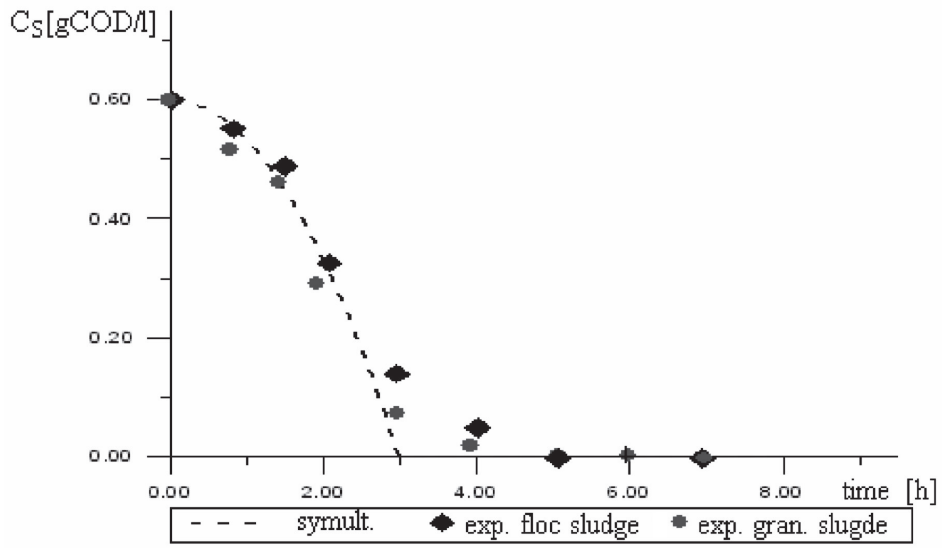


Figure 7: Substrate concentration versus time.

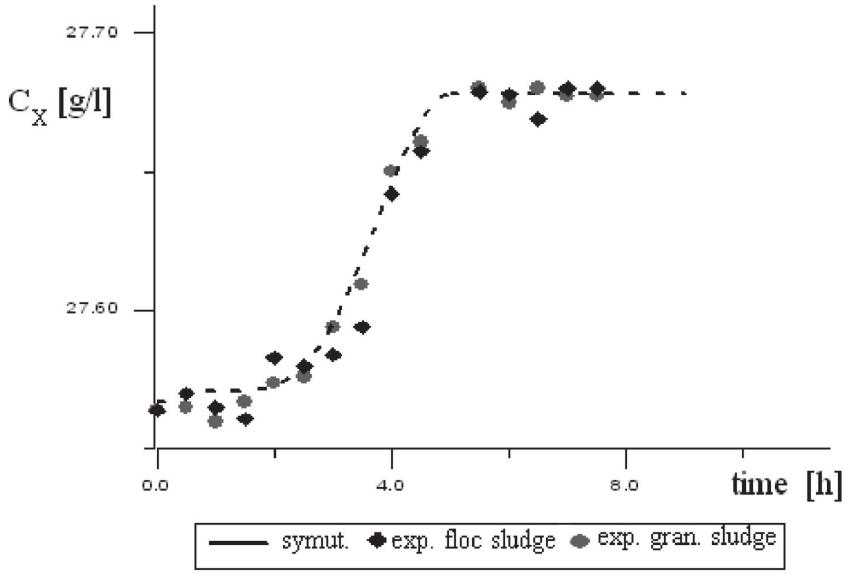


Figure 8: Biomass concentration versus time.

one is related to biomass (x) and the other to cell numbers (N). For cell biomass, growth can be considered as an autocatalytic reaction. Therefore, the rate of growth is dependent on the biomass concentration, i.e. catalyst, that is present at any given time. Also, the generation time recorded during microbial growth is in reality an average value, as the cells will not be dividing at exactly the same rate. At anyone time there are cells at different stages of their cell cycle. This is termed asynchronous growth.

During fermentations the population of micro-organisms goes through several distinct growth phases: lag, acceleration, exponential growth, deceleration, stationary and death. In the lag phase virtually no growth occurs and the microbial population remains relatively constant. Nevertheless, it is a period of intense metabolic activity as the microbial inoculum adapts to the new environment. When cells are inoculated into fresh medium they may be deficient in essential enzymes, vitamins or cofactors, etc., that must be synthesized in order to utilize available nutrients, prior to cell division taking place. Intermediate product concentration also changes with time is shown in Figure 9 and the final product concentration versus time is shown in Figure 10.

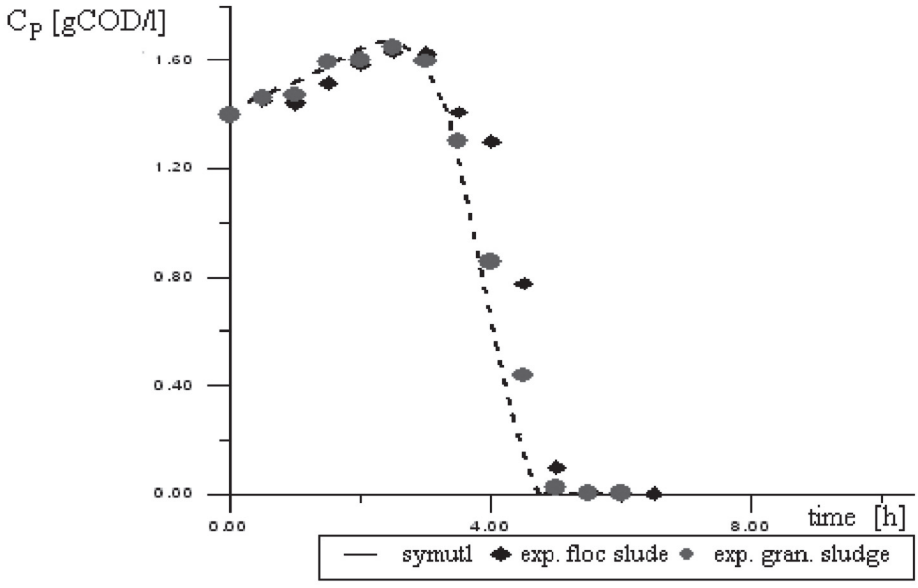


Figure 9: Intermediate product concentration versus time.

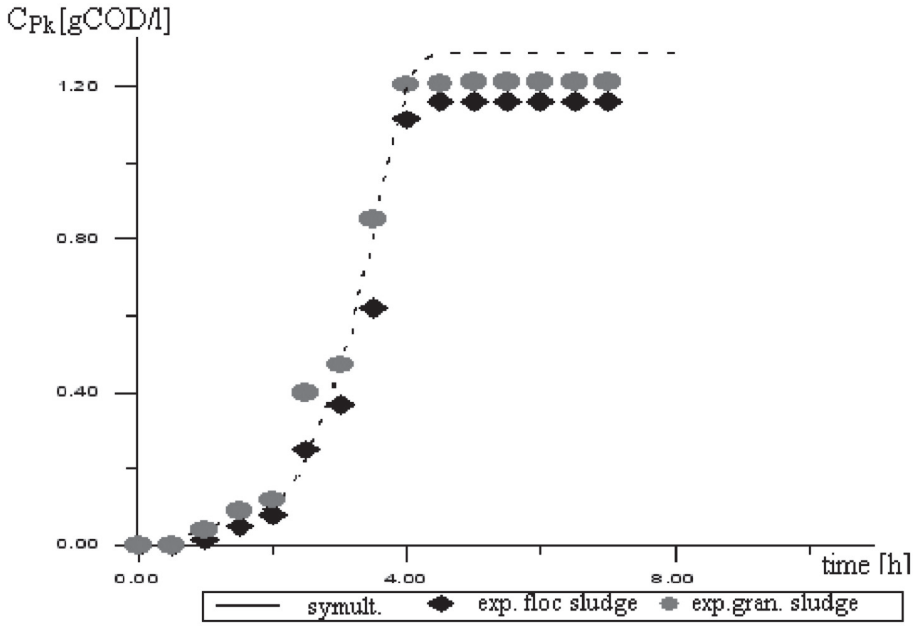


Figure 10: Final product concentration versus time.

Conclusion

The results of the study lead to the following conclusions.

(a) By proper maintenance of required alkalinity, the BOD reduction can be increased.

(b) The maximum BOD reduction was obtained at an applied organic load of 0.80 kg BOD/m^3/day.

(c) The BOD reduction was more than 94% which could be due to the proper maintenance of alkalinity and volatile acids in the digester.

(d) Due to pH correction, the alkalinity level in the waste was boosted up and this helped in maintaining adequate buffer capacity in the digester to neutralize the volatile acid.

(e) Compared to other alkalis, it appears to be an increase in biogas production during anaerobic digestion by the use of $MgCO_3$ in the system.

(f) The substrate utilization rate increase may be attributed to continued increase in metabolic activities caused by cell growth.

(g) The rate constant values confirm a first order reaction.

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Calendar of Events

3rd International Perspective on Current & Future State of Water Resources & the Environment
5 to 7 January 2010
Chennai, Tamil Nadu, India
Website: http://content.asce.org/conferences/india2010/index.html
Contact name: Dr. Chandra S. Pathak
Organized by: ASCE EWRI

National Seminar on Soil Salinity and Water Quality
19 to 21 January 2010
Karnal, Haryana, India
Website: http://www.cssri.org
Contact name: Dr. Pradip Dey, Organizing Secretary
Organized by: Indian Society of Soil Salinity and Water Quality and CSSRI (ICAR)

National Conference on Conservation of the Wetlands - A Multidisciplinary Approach
28 to 29 January 2010
Alappuzha - the Venice of the East, Kerala, India
Website: http://www.stjosephcollegeforwomen.com/php/newsUpdates.php?id=10&linkid=21
Contact name: Dr. Marykutty Abraham
Organized by: Department of Botany, St.Joseph's College for Women, Alappuzha, ATREE

Water Scarcity
1 to 11 February 2010
Salzburg, Austria
Website: http://www.edu-zgis.net/ss/waterscarcity2010
Contact name: Antonia Osberger
Organized by: Centre for Geoinformatics

Improving Efficiency in Water Systems Seminar
2 February 2010
Limassol, Cyprus
Website: http://www.bentley.com/efficiency2010
Contact name: Perrine Parrod
Organized by: Bentley

EcoForum 2010
23 to 24 February 2010
Sydney, NSW, Australia
Website: http://www.ecoforum.net.au/2010
Contact name: Margaret Bates

2010 International Conference on Environmental Science and Development (CESD 2010)
26 to 28 February 2010
Singapore
Website: http://www.iacsit.org/cesd/index.htm
Contact name: Conference Secretary

Coasts, Marine Structures & Breakwaters 2010
3 to 4 March 2010
Sydney, NSW, Australia
Website: http://www.marinestructures.com.au
Contact name: Chris Archer
Organized by: IQPC Australia

Sustainable Water Resources Management and Impact of Climate Change
5 to 6 March 2010
Hyderabad, Andhra Pradesh, India
Website: http://www.bits-hyderabad.ac.in/swrm/National_conference/
Contact name: Prof. K. Srinivasa Raju and Dr. A.Vasan
Organized by: BITS-Pilani, Hyderabad Campus

The Second International Conference on Integrated Water Resources Management and Challenges of Sustainable Development (GIRE3D)
24 to 26 March 2010
Agadir, Morocco
Website: http://www.fsa.ac.ma/gire3d
Contact name: Lhoussaine Bouchaoui
Organized by: Moroccan Committee of International Association of Hydrogeologist (CM-IAH)

International Drought Symposium
24 to 26 March 2010
Riverside, California, United States
Website: http://cnas.ucr.edu/drought-symposium/
Contact name: Dr. Ariel Dinar