Brewery Wastewater Treatment Using Aerobic Sequencing Batch Reactors with Mixed Culture Activated Sludge

by

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Abstract

Laboratory-scale aerobic sequencing batch reactors, in both suspended-growth and attached-growth modes, were used to study the treatment of brewery wastewater. A Ringlaces material was selected and employed for the attached-growth reactors. Experiments were conducted employing a wide range of hydraulic retention times, from 0.56 to 6.06 days. The experimental results demonstrated that brewery wastewater could be successfully treated using both suspended-growth and attached-growth aerobic sequencing batch reactors. Treatment efficiencies in terms of the removals of total organic carbon (TOC), the five days biological demand (BOD_5), chemical oxygen demand (COD), and suspended solids (SS) were consistently maintained over 90%, with the suspended-growth reactors performing significantly better than the attached-growth reactors.

As the results of these experiments demonstrated that the performance of suspended-growth SBRs was superior to that of attached-growth SBRs, only the suspended-growth SBR system was selected to study the optimal conditions of HRT and loading rate. The results showed that the maximum removal of TOC and SS could be reached at the optimal of HRT and loading rate. The removal of TOC was more sensitive to variations in the HRT than to variations in the loading rate; however, the effect of loading rate was dominant in the removal of SS compared to the effect of the HRT.

The pH remained relatively constant during the aeration stage. The dissolved oxygen concentration changed as aeration proceeded. This may be related to TOC

degradation and microbial activity. A lower sludge production rate was observed in the aerobic suspended-growth SBRs.

Table of Contents

Materials and Methods

Conclusions and recommendations

List of Tables

List of Figures

List of Abbreviations

ANOVA: Analysis of variance

BOD_5:   Biological oxygen demand in five days

COD: Chemical oxygen demand

CORR: Correlation analysis

DO: Dissolved oxygen

LSD: Least significant difference

MLSS: Mixed liquor suspended solids

HRT: Hydraulic retention time

SBR: Sequencing batch reactor

SS: Suspended solids

TKN: Total kjeldahl nitrogen

TOC: Total organic carbon

VSS: Volatile suspended solids

VS: Volatile solids

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Introduction

1.1 General characteristics of brewery wastewater

Brewery plants produce large quantities of highly polluting wastewater rich in organic substances. As the scale and production of the brewing industry increases, the amount of wastewater increases substantially, resulting in increasing pollution problems in the environment. Due to wide variations in its strength (in terms of COD, BOD_5, and TOC), pH and the amount of wastewater discharged, brewery wastewater tends to be very difficult to treat. In view of this situation, there is a need to develop a technology which is capable of efficiently treating the increasing volumes and strength of wastewater from brewery plants.

The wastewater discharged from breweries is generally a combined effluent comprising discharges from various sources within the plant. The fermented liquor is the final product in the brewing process. Wastes arise from the separation of grain residues, from spent-hops and yeast from the fermentation processes, from spillage, from possible spoilage of the beer, from fillers as well as from packaging and from washing wastewater. The wastewater production rates from the brewing and packaging sections vary independently of one another. While the packaging process produces a high flow, high pH, weak waste composed primarily of spilled beer and caustic bottle cleaning solutions, the brewing process produces a low flow, neutral pH, and high strength alcohol-carbohydrate-protein waste.

A continuous monthly monitoring of the effluent from a brewery plant showed considerable variation in general wastewater characteristics in terms of biological oxygen

demand (BOD_5), chemical oxygen demand (COD), and solids concentration. As described in Table 1.1, total COD varied from 87 mg/l to 6550mg/l, and the concentration of suspended solids varied from 16mg/l to 1162mg/l. The ratio of soluble BOD_5 to total BOD_5 was about 0.91, which implied that most of the biodegradable materials were in soluble forms. One important characteristic of brewery waste is its fluctuation in flow and quality at night and weekends, compared with the average working day flow. The fluctuations in the quantities of wastewater discharged from a local brewery is depicted in Figure1.1.

Table 1.1 The general characteristics of brewery wastewater.

Total BOD_5	41-4260 mg/l
Soluble BOD_5	34-3890 mg/l
Total COD	87-6550 mg/l
Soluble COD	37-4830 mg/l
Total Suspended Solids	16-1380 mg/l
Volatile Suspended Solids	11-1230 mg/l
pH	6.1-9.1

The pH of the wastewater from various processes within the plant was neutralized in a pH tower within the plant. This ensured that the pH did not change significantly. Since brewery wastewater has a poor buffering capacity (Cronin, 1996; Cronin and Lo, 1998), hydrolysis and anaerobic activity usually reduce the pH. The pH tends to drop from 10 to 4 within a day at room temperature, and it will drop from 10 to 5 within 3-4 days in a walk-in cooler at temperature approximately 4 °C. Due to a preponderance of carbonaceous matter it tends to be relatively short of nitrogen-nutrients. Slight seasonal temperature variations in the wastewater ranged between 19 °C in the winter and 31.4 °C in the summer.
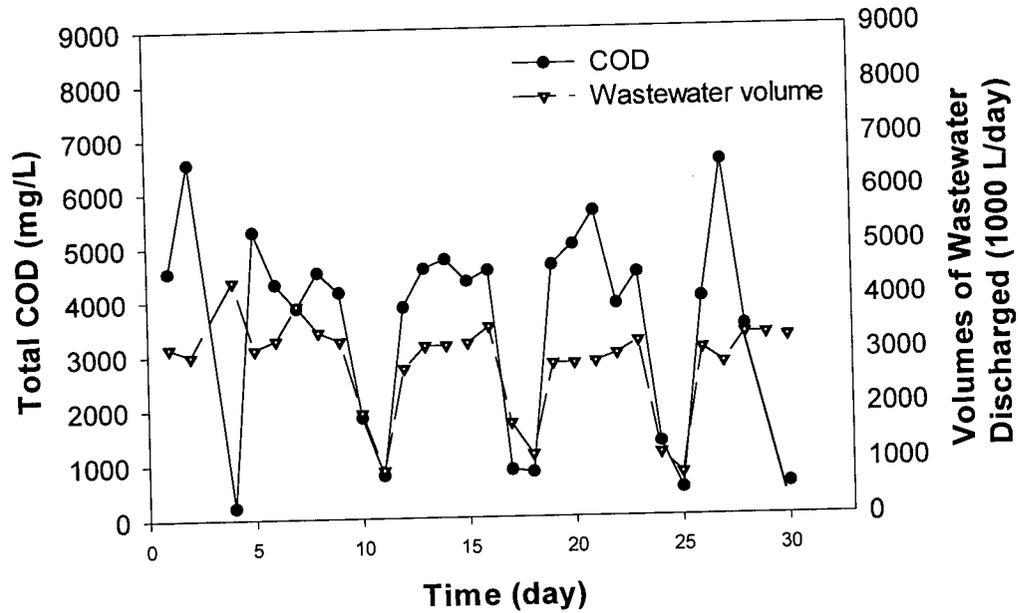
Figure 1.1 Monthly monitoring of brewery wastewater

Data for the Figure 1.1 were obtained from a monthly continuous monitoring of brewery wastewater in a local brewery. Samples were collected randomly once a day. The sampling month was July, 1997.

1.2 Objectives

The objectives of this study were:

- to start-up aerobic sequencing batch reactors for brewery wastewater treatment by using mixed culture activated sludge;

- to compare treatment efficiencies of suspended-growth and attached-growth in the aerobic SBRs;

- to investigate the effects of variations in loading rate and HRT on suspended-growth aerobic SBRs; and

- to distinguish the relationships of TOC degradation, the concentration of dissolved oxygen (DO), and the pH with aeration time in suspended-growth SBRs.

Literature Review

2.1 Previous research on brewery wastewater treatment

Due to its high organic content and high biodegradability, brewery wastewater is ideally suited to biological treatment. All treatment methods basically involve the conversion by microorganisms of fairly complex stable/unstable organic compounds to carbon dioxide and water. Biological treatment results in the removal of BOD_5, the coagulation of nonsettleable colloidal solids, and the stabilization of organic matter. Most of the previous research involving brewery wastewater treatment used anaerobic treatment systems.

2.1.1 Anaerobic processes in brewery wastewater treatment

It has been widely recognized that anaerobic treatment of high strength industrial wastewaters can be competitive with conventional aerobic treatment processes. Among those anaerobic treatment processes, anaerobic digestion, in particular, has been suggested as a suitable process in brewery wastewater treatment (Sax, 1985; Ince, et al., 1986; Huang, et al., 1989; Lo et al., 1989; Ware, et al., 1989; Anderson, 1991; Strohwald, et al., 1992; Tanemura et al., 1992; Liang, et al., 1993; Chung, et al., 1993; Cronin,1996).

The treatment of brewery effluent by the anaerobic digestion - ultrafiltration (ADUF) process has been studied on a laboratory scale (Strohwald, 1992). The results showed that COD reductions of 96%-99% were possible at a loading rate of 15 kg COD m-3.d-1 and a hydraulic residence time of 0.5-0.8 days. The COD values of the UF

permeate (final effluent) were generally below 100 mg/l for an 80 day test period. Since the brewery effluent showed a nitrogen deficiency, the addition of urea was found to result in more stable and improved digester performance. Tanemura et al. (1992) investigated the operation conditions for anaerobic treatment of wastewater from breweries, and they compared the efficiency of single- and double-anaerobic fluidized-bed reactors (AFBR). They found that the double-AFBR process was more advantageous in obtaining treated effluent which could be discharged into rivers.

The anaerobic pretreatment of brewery wastewater on an industrial scale has been reported by Sax (1985). A Biothane wastewater treatment system employing upflow anaerobic sludge blanket (UASB) technology has been used and has been in nearly continuous operation at a brewery since late 1981. Soluble COD removal efficiencies of about 90% are routinely achieved at volumetric loading capacities of 5 to 10 kg COD per cubic meter of digester volume per day and a hydraulic retention time within the digester vessel of about 6 hours. The methane purity of the generated biogas approaches 80%, and the methane yield is close to the theoretical stoichiometric quantity of 0.35 cubic meter per kg of COD removed. The system has been shown to perform consistently despite fluctuations in day-to-day COD loading by nearly a factor of three. Normal anaerobic biomass growth within the digester occurs, and sludge losses due to washout are trivial. However, several problems appeared in this system; for example, filamentous bacteria were growing luxuriantly, and some ancillary equipment for odor control and upstream solids removal needed to be improved. The growth of filamentous bacteria was resolved by upstream acidification to insure consumption of dissolved oxygen within the wastewater.

The anaerobic process potentially has many advantages. It produces methane gas which may be burnt as fuel resulting in the production of carbon dioxide and water. In addition, anaerobic fermentation produces far less sludge than aerobic processes. Its wide applications has hindered by the inconvenience and difficulty of sufficiently intensifying the anaerobic process at ambient temperatures and the inability to adequately control the fermentation leading to methane formation. In addition, the resultant methane gas can be nauseating if leaked to the atmosphere. Furthermore, anaerobic treatment is generally considered as a pretreatment and usually requires post-treatment to meet effluent standards. In the specific case of the anaerobic treatment of brewery wastewater, the pH fluctuation, sludge bulking, and longer reaction times are among the problems encountered.

2.1.2 Aerobic treatment of brewery wastewater

When the organic strength of a wastewater is not too high, conventional aerobic biological treatments are often a cost-effective form of treatment. In the case of brewery wastes, aerobic treatment has in the past proven to be successful on an industrial scale, as demonstrated by the deep shaft treatment system at Molson Brewery in Barrie, Ontario (Le Clair, 1984). This deep shaft process was capable of producing an effluent having an average $TBOD_5$ and TSS less than 50 mg/l. It was claimed that the potential of deep shaft process would reduce energy and space requirements, have lower capital costs and reduced sludge production compared to conventional technology. However, the operation suffered from several mechanical problems such as the failure of the main downcomer section. In the case of a poorly flocculating sludge, an increased rotifer concentration and

7

nearly zero protozoa levels were seen during periods when yeast levels in the waste were high. Both protozoa and rotifer concentration dropped to low levels when beer levels in the waste were high.

The high rate aerobic treatment of brewery wastewater using the Jet Loop reactor has been reported by Bloor et al. (1995). The use of a jet aeration system for the biological treatment of wastewater is becoming more commonplace as a means of combining efficient oxygen transfer with high turbulent mixing. In their studies, a loading rate of 50kg COD/m^3/d was achieved with 97% COD removal for a period of 5 weeks. Although the settleability was found to be acceptable, non-flocculating motile bacteria caused the effluent to be cloudy and to have a high suspended solids concentration in the order of 200-350 mg/l.

A Two-stage unitank system has also been developed for the treatment of brewery wastewater (Eyben, 1985). After a series of preliminary treatments including screening, grit-removal, no primary settlement, and eventually buffering, the wastewater is treated in a high-loaded combined aeration sedimentation stage. The BOD$_5$-reduction is about 80-85%. The partial purified water then flows by gravity to a second low-loaded combined aeration sedimentation stage where the residual BOD$_5$ is removal to obtain the high-quality effluent resulting in more than 98% BOD$_5$-reduction. This two-stage unitank system had better process performance in terms of high-treatment efficiency and control of sludge bulking. In addition it is a simple and reliable process and is easily controlled by microprocessor. Its flexible operation allows for temporary operation at half capacity and quick restoration to full capacity. It also allows for temporary operation as two high-loaded one-stage systems (during periods of peak production or heavy rainfall) thereby

conserving a treatment efficiency of 80-85% BOD_5-reduction. Lower capital and operating costs are also an advantage of the two-stage unitank system. Eyben (1995) also studied the use of the unitank system in the biological removal of nitrogen and phosphorus from brewery waste water. They reported biological N and P removal efficiencies of 90-99 and 97%, respectively, using this system.

Sequencing batch reactor systems are receiving increasing use in the treatment of municipal, industrial and agricultural wastewater (Irvine and Busch, 1979; Lo et al., 1986). However, reports on the application of the SBR process to brewery wastewater are scarce. The performances of an SBR operated in the conventional mode and an SBR using an alternative mode called the contact-stabilization mode were compared in the treatment of brewery wastewater by Yu et al. (1997). The results showed that this new contact-stabilization operational mode, based on the concept of rapid uptake of organic matter by biomass in a short retention time and subsequent regeneration of biomass after decant, had no negative influence on organic material removal efficiency and biomass settleability. A new operating scheme for a three-tank SBR system was proposed in their study. This alternative scheme was able to expand the three-tank SBR system's capability of withstanding inflow variations.

2.1.3. Other treatment methods of brewery wastewater

As an alternative to the biological treatment of brewery effluent arising during the fermentation process, a recovery process for yeast and ethyl alcohol was analyzed in terms of its economic implications (Wysocki, 1973). Such an effluent can contain up to 16% yeast and 2.3% ethyl alcohol results in a BOD_5 of 200 g/l. In economic terms, such a

9

recovery process for yeast and alcohol seems to be the most reasonable solution to this particular effluent problem. An aerobic spore-forming *Bacillus sp.,* isolated from a hot spring, was found to produce hydrolytic extracellular enzymes when cultured on opaque brewery wastewater supplemented with defatted soy, spent yeast, and malt flour (Zvauya, 1996).

Fe (1974) reported on the efficiency of the biofiltration of brewery waste water treatment. Advantages claimed included high efficiency, low construction costs, fast start-up, insensitivity to environmental conditions, low space and personnel requirements, and production of an easily flocculated protein-rich sludge suitable for sale as animal feed.

The treatability of high strength brewery wastewater with stabilized refuse was studied by Fan (1990). A column with a surface area of 790 cm^2 and effective height of 105 cm was used for the study. The column was filled with stabilized refuse compacted to an average density of 500 kg/m^3. High strength brewery wastewater with a COD of 6000 mg/l was homogeneously trickled over the top of the refuse at flow rates of 8, 16, 24, and 36 l/day. After six month intensive study, it was demonstrated that the stabilized refuse method was effective in the treatment of the brewery wastewater. The COD was reduced to as low as 60 mg/l after the wastewater had trickled through the 90 cm refuse layer which was equivalent to a removal efficiency of 99%. The variations in major parameters such as pH, alkalinity, and volatile fatty acids gave a strong indication that acidogenesis occurred quickly in the first 15 cm. As the flow rate increased, acidogenesis occurred deeper in the column and its recovery slowed. The oxidation reduction potential

(ORP) dropped to as low as –290 mV in the refuse column, indicating the anaerobic condition of the system (Fan, 1990).

2.2 Sequencing batch reactor

The mass of contaminants present in domestic and industrial wastewater, in leachates and groundwater, and in soils generally varies with time and space. These natural and sometimes severe variations are coupled with the uncertainties associated with direct exposure to the environment. However, despite such unsteady-state behavior, facilities used for the removal of contaminants are often designed with the potentially unrealistic expectation that they can be operated as steady-state systems (Irvine et al., 1997). The SBR technology may be regarded as one of a number of methods which can be operated periodically and as a controlled unsteady-state system.

2.2.1 Operation of SBR

An SBR biological treatment unit operates periodically in a typical cycle of five phases: FILL (inflow of wastewater), REACT (aeration), SETTLE (quiescent sedimentation of biomass and solids), DRAW (outflow of treated effluent) and IDLE. Figure 2.1 illustrates the scheme of a typical SBR process cycle. The filled phase and drawn phase are operated within a defined period of time. After completion of filled phase, variations in the influent of the treatment plant no longer have any effect on the process taking place in the reactor just filled.

Before filling, the reactor contains an active and sizable microorganisms population which will biodegrade the influent wastewater. At start-up, microorganisms

11
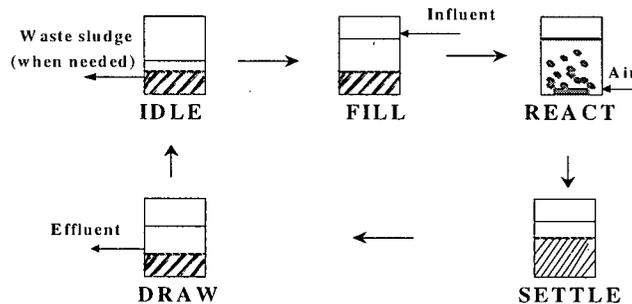
Figure 2.1 The scheme of SBR operation.

must be seeded into the reactor from a suitable source. Once operating, however, the biomass remains in the reactor from cycle to cycle. The cyclic nature of the SBR process allows control over the dilution of operating conditions including the air supply and mixing, and it is thus possible to design a series of specific states in order to achieve a particular set of biochemical reactions on given waste materials.

2.2.2 The applications of SBR in wastewater treatment

In recent years, SBRs have emerged as an innovative technology in the wastewater treatment systems. It has been found to be an efficient and flexible method for treating various dilute wastewater (Alleman et al., 1985; Decreon et al., 1985; Tam et al., 1986; Imura, et al., 1993). Since SBRs are time-oriented, not space-oriented, fill/reaction ratios, aeration periods, and mixing cycles may be altered to accommodate specific operating conditions and to yield desired results. SBRs are therefore uniquely suited to wastewater treatment applications which are characterized by low and/or

intermittent flow conditions. Furthermore, advances have occurred in sludge bulking control technologies using selectors mechanisms. Because SBRs have the flexibility to incorporate many of these selectors mechanisms in their operation, they are also suited to applications under high organic/low nitrogen concentration conditions (Sheker et al., 1993).

It has been reported that an SBR system, operated in a fill-and-draw mode under sequential anoxic/aerobic conditions in a single tank, yielded a high combined removal rate of organic carbon and nitrogen compounds (Fernandes et al., 1991, 1994). An anoxic fill sequence, rich in exogenous organic carbon, favored denitrification and as a result oxidized nitrogen levels dropped. In the aerated react phase the ammonia accumulated during fill was oxidized to NO_2-NO_3-N. The nitrifiers were not inhibited by the anoxic operation.

Some studies have also described SBR processes consisting of two or more tanks. The tanks were operated in a fill-and draw mode, which is the same as uni-tank system. One tank accepts the incoming wastewater while the others are in reaction, settling, draw-down, or idle phases. When the first tank is full, the incoming wastewater stream is diverted to a second tank which has been drawn and is in a standby phase ready to accept wastewater (Irvine et al., 1988; Ketchum et al., 1979). Ketchum et al. (1979) observed that a highly variable oxygen demand was exerted on an SBR system. They stated that the required aeration rate increased from a level needed for endogenous respiration in the standby phase, when the substrate concentration and liquid volume were both low, to a peak at the end of the filling cycle. During the reaction phase the required aeration rate

was dropped to a level needed for endogenous respiration and then shut off completely to allow settling and draw down to the minimum level needed to contain the settled solids.

Novel approaches have also been developed based on sequencing batch reactor concepts. One was the sequencing batch biofilm reactor (SBBR) technology. Hirl and Irvine (1997) used anaerobic SBBR to dechlorinate reductively PCE (perchloroethylene). The ASBBR was a periodically operated up-flow packed column reactor. It was operated on a cycle consisting of three periods: FILL, REACT, and DRAW, and was constructed from glass columns filled with acid washed pea gravel as a support matrix. The consortium of microbes which can decholorinate was enriched by this ASBBR, and the reductive decholorination always occurred in the presence of methanogenesis.

The efficiency of an SBR operation is also affected by factors such as nutrient levels, temperature, and fill strategies. It has been proven that temperature influenced nitrification and denitrification in anaerobic SBR (Fernandes, 1994; Schmit et al., 1994). It has been also proven that higher temperature exert a positive influence on the overall performance of the SBR in the range of 5-21 °C, and the process performance seriously deteriorated at 5 °C (Fernandes, 1994). The effects of anoxic and oxic fill strategies on SBR performance under nitrogen (NH_4Cl as the nitrogen source) deficiency and rich conditions were evaluated using glucose as the sole substrate (Sheker, 1993). The performance was evaluated on the basis of substrate removal, sludge settleability, supernatant suspended solids, and reactor biomass concentration. Substrate removal efficiencies were found to be independent of the fill strategies adopted under all conditions tested. The incorporation of anoxic selector environment failed to prevent the development of bulking sludge under conditions of nitrogen deficiency, thereby resulting

in a gradual depletion of reactor biomass. Under nitrogen rich conditions, the sludge settleability improved significantly when an anoxic fill strategy was adopted. Furthermore, suspended solids readings taken at the end of the settling period were greater with anoxic fill than with oxic fill, indicating that the latter discourages the growth of dispersed bacteria.

2.2.3 Advantages of an SBR system as a wastewater treatment

Compared with continuous systems, SBR systems are more dynamic and flexible in terms of operation and more advantageous kinetically (Irvine et al., 1979, 1989, 1997). The SBR process covers a range from feast to famine during the reaction cycle and the different aerobic/anoxic/anaerobic conditions imposed. Since SBRs impose a diverse array of operating conditions and selective pressures, they can be used as a versatile tool for the enrichment of specific consortia and the induction of desired metabolic pathways. By adding the system's own periodicity of forcing function, the potentially negative impact of those forcing function associated with variations in contaminant concentration, and other environmental uncertainties, can be migrated (Irvine et al., 1997).

Research concerning sequencing batch operations indicated that the SBR concept is a viable and economically attractive alternative to the conventional continuous flow activated-sludge process in BOD_5, SS and nitrogen removal, as well as in the chemical precipitation of phosphorus. The dynamic and flexible nature of SBR systems allows ample room for expansion and operational adjustments at minimal costs.

The advantages of an SBR wastewater treatment system are summarized below:

- Less react volume because both aeration and settling are in the same tank;

- Batch discharge of only treated wastes meeting effluent limitations (this is possible by monitoring the wastes and providing additional treatment if its quality is poor);

- High oxygen transfer efficiency;

- Facilitated design of a series of specific operating states;

- Anaerobic periods enhance denitrification and nitrogen removal, control for filamentous organisms and reduce power consumption; and

- Cost effective

Materials and Methods

3.1 Sources of brewery wastewater and activated sludge

Brewery wastewater was collected weekly from a local brewery and stored in a cold room at 4 °C. The wastewater was allowed to reach ambient room temperature (20 ± 2 °C) before being fed into the SBRs. An acclimation period was provided for the microorganisms to adjust to the new environment. The characteristics of brewery wastewater are depicted in Section 4.1.

A mixed culture activated sludge, taken from the municipal wastewater treatment pilot-plant at the University of British Columbia campus, was used as seed for all the SBRs. Before seeding the reactors, the mixed culture activated sludge was settled, and only the condensed sludge was used. The range of BOD_5 of the activated sludge used to seed the reactors was from 6.66 to 15.6 g/l, and the average of suspended solids was about 4.93 g/l.

3.2 Equipment

Four lab-scale reactors made of acrylic plastic pipe, with 62 cm in height and 19 cm in inside diameter were used in these experiments. The total volume of each reactor was 15 l, and the working volume of each reactor was 12 l.

A computer system with LABTECH Control software (Laboratory Technology Co., 1994) was used to control the sequencing cycle as well as to monitor the pH and dissolved oxygen concentration in the reactors. A PC-711 board was installed in the computer and connected with a relay box, which was linked to the pumps and aerators.

Signals coming from computer switched the pumps and aerator on and off. Other instruments used in this research are listed in Table 3.1.

Ringlaces was selected to set up immobilized growth aerobic SBR due to its ability in rapid entrapment of microbial biomass. This Ringlace is a synthetic fibrous rope product with loops approximately 1.5 cm long protruding from the center all along the length of Ringlace. It is manufactured from polyvinylidene chloride fiber 100 microns in diameter. It was reported that the material is water resistant and chemically very stable. The fibers are knitted and twisted into individual strands. The flexible fibrous loops are supposed to provide a large surface area for the attached growth and in combination with the swaying motion of the flexible rope tend to improve shedding action to prevent clogging in the process (Setter, 1995).

Two peristaltic pumps (each with four pump heads) were used to feed influent and withdraw effluent from the four reactors. Therefore, the same flow rate of feeding and discharging were carried out for the four reactors. Aeration was controlled by adjusting the aerator setting so that the air supply was kept at the same amount for these four reactors. The set-up of a suspended-growth reactor, and a reactor with Ringlace (attached-growth) is shown in Figure 3.1.

Table 3.1. Apparatus used in the experiments.

Apparatus	Quantity	Description
Reactor	4	Acrylic plastic colume, 19 cm x 62 cm. Working volume: 12 l
Computer	1	386 IBM
Input/Output control card	1	PCL-711
Peristaltic pump	1	Cole-Parmer, 6-600 rpm
Peristaltic pump	1	Masterflex, 1-100 rpm, Model #661063
Pump speed controller	2	Masterflex, Model #7553-71, 50/60 Hz, 3amp
DO probe	2	Oxyguard, PT4 system Inc.
DO meter	1	PT4 oxygen meter, PT4 system Inc.
pH probe	2	VWR scientific (Ag/AgCl), Cat. #34105-023
pH meter	1	Good-Digital , 201 ATC
Aerator	2	Maxima/Optima, Rolf C. Hagen Inc.

(a). Effluent pump
(b). Influent pump
(c). Control system
(d). Air stone
(e). Aerator
(f). Ringlace
(g). Reactor

(1)

(2)
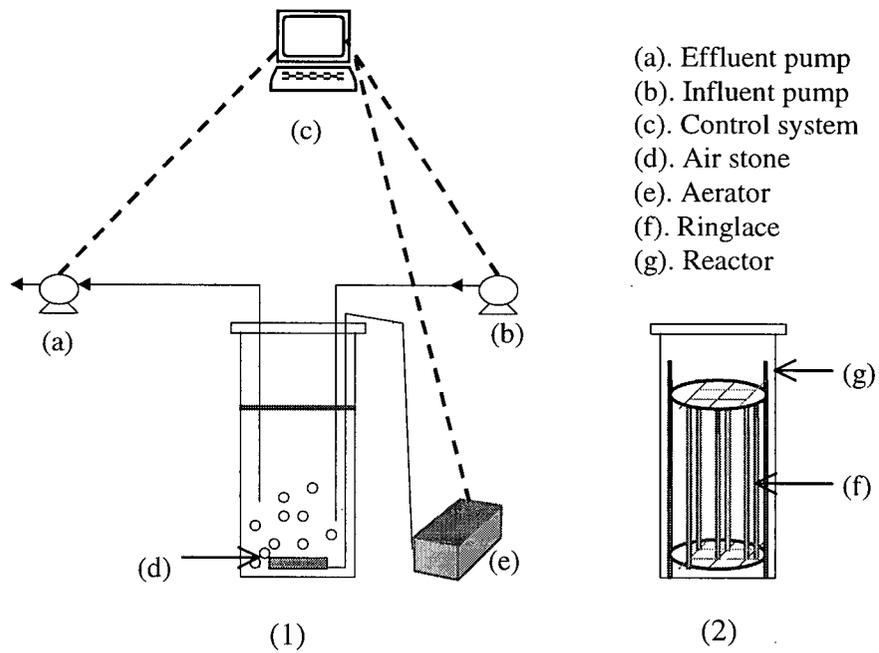
Figure 3.1. Diagram of the reactor set-up: (1) a suspended-growth reactor; (2) a attached-growth reactor (i.e. reactor with Ringlace).

3.3 Experimental Design

3.3.1 The aerobic SBR start-up and operation scheme

Four reactors were seeded with equal volumes of condensed activated sludge (2.5 l), and anoxically fed with brewery wastewater. The activated sludge was allowed to acclimate after innoculation in brewery wastewater for 3 to 4 weeks. Sludge retention time (SRT) was fixed at 21-28 days through the experiments. An anoxic fill strategy was adopted in order to prevent the development of bulking sludge (Sheker et al., 1993).

The reactors were operated following the basic SBR operation scheme as described in Figure 3.2. Sequencing time length of cycles was set according to the purposes of the experiments. During the fill period, 4 l of feed (brewery wastewater) was introduced to each of the reactor and the total working volume was brought from 8 to 12 l. Aeration was discontinued during the settle period, and sludge was allowed to settle under relatively quiescent conditions. In discharge stage, 4 l of the clarified effluent was withdrawn and the liquid volume was decreased to 8 l. The idle period was set as a time to prepare and maintain the reactor for the next cycle.

To study parameters related to the aerobic SBR start-up, two reactors were employed with the suspended-growth mode. One of them had a mixer installed and the other reactor was run without a mixer. The hydraulic retention time (HRT) was maintained at 1.56 days (including 12 hours for aeration, 20, 4, 3, and 3 minutes for settling, discharging, idle, and feeding time, respectively) throughout the start-up stage.

21

Waste sludge (when needed)

Influent (4 l)

Air

IDLE
(3 min)

FILL
(3 min, anoxic)

REACT
(4-48 hrs)

Effluent (4 l)

Working volume: 12 l
SRT: 21-27 days

DRAW
(4 min)

SETTLE
(20 min)
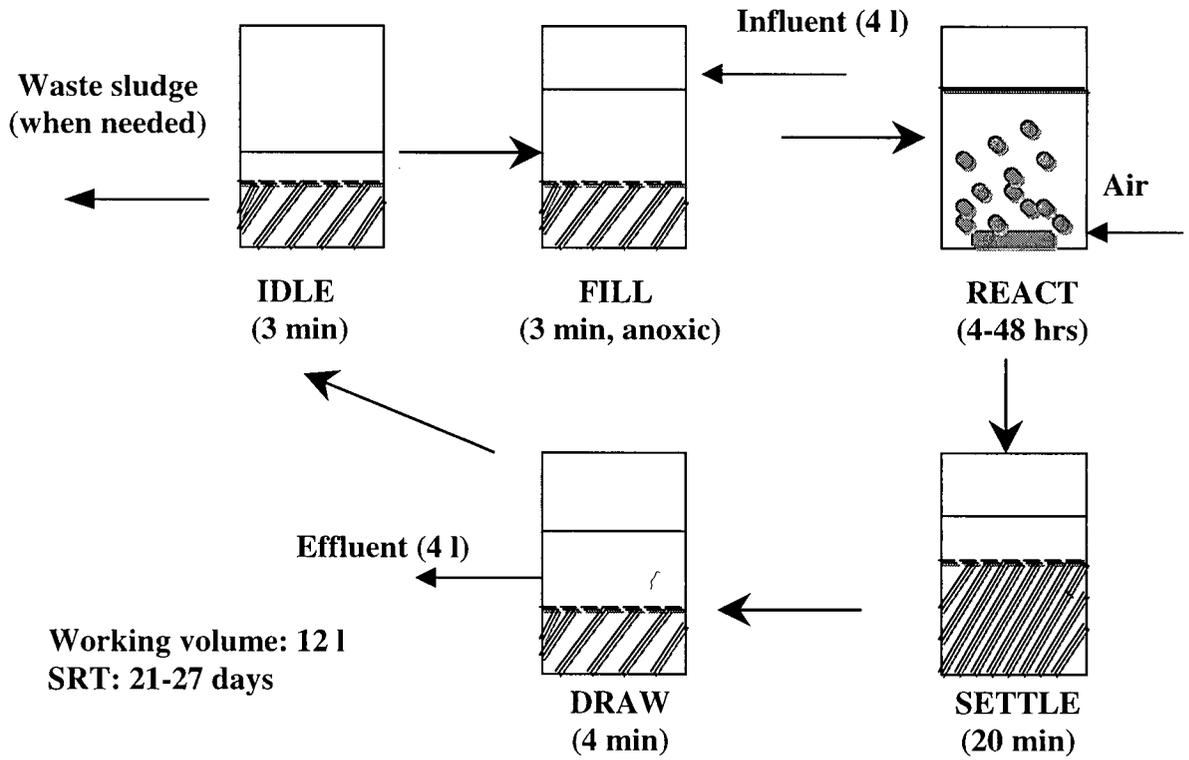
Figure 3.2 The operation scheme of SBR.

3.3.2 Studies on comparison of treatment efficiencies under suspended-growth and attached-growth conditions

In this study, two of the four reactors were used for suspended-growth, and the other two reactors were used for attached-growth.

To set-up the attached-growth reactors, precisely tensioned strands of Ringlace, 5 cm apart, were attached between two horizontal plastic support rods. The support rods were then affixed to rigid frames installed in the reactors. The support rods were also spaced 5 cm apart, center to center, thus creating a matrix of Ringlace strands. Figure 3.3 is a photograph showing the set-up of Ringlace media. The operations of these reactors were the same as those of the suspended growth reactors.

From our literature review and preliminary experiments, we found that the performance of these aerobic SBRs may be limited by factors such as inadequate oxygen transfer, settling of biomass, stability under different loadings, and hydraulic retention time. In this study, the HRT and loading rates were varied over a wide range of wastewater concentrations. Experiments were carried out with HRTs over a range of 0.56, 0.81, 1.06, 1.56, 3.06, and 6.06 days (Table 3.2). During these experiments, the sequencing cycles were adjusted according to the changes of aeration time, but the time for FEED, SETTLE, DRAW, and IDLE, was fixed at 3, 20, 4, and 3 minutes, respectively. Therefore, the difference of HRT was mainly due to the aeration time, and the time courses of the other stages had no effects on HRT.
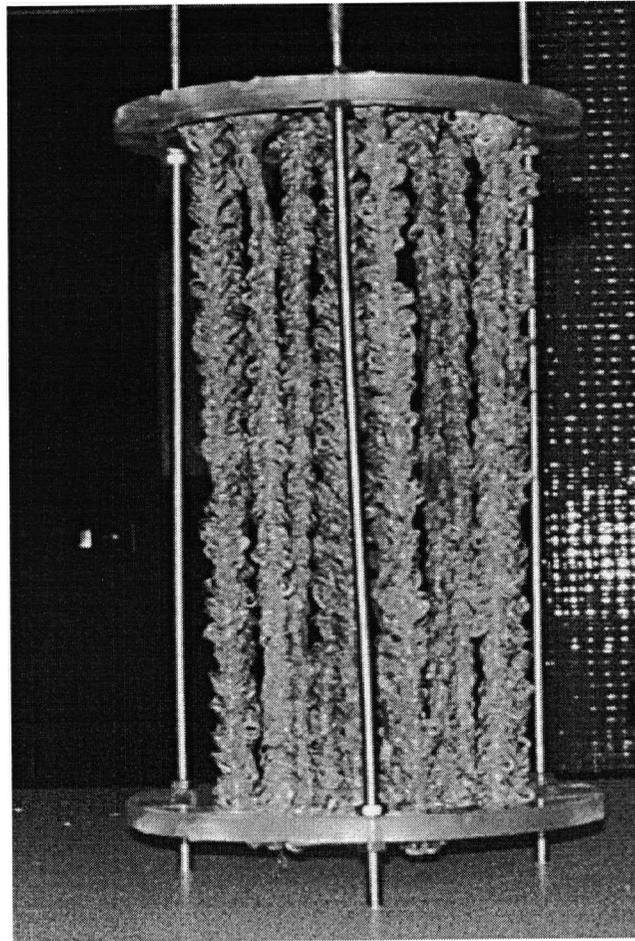
Figure 3.3 The photograph of a Ringlace.

Due to the characteristics of the raw wastewater, variations of the influent concentration under each HRT would result in changes of the loading rate. All other experimental parameters, such as anoxic feeding, mean cell residence time (MCRT), influent and effluent flow rates, were maintained the same way in both suspended-growth and attached-growth reactors. The operations of the SBR were the same as described in Section 3.3.1.

Table 3.2   Time courses of aerobic SBR under suspended growth

and immobilized growth condition.

Run #	Aeration time (hrs)	Settling time (min)	Discharge time (min)	Idle time (min)	Fill time (min)	HRT (day)
#1	48	20	4	3	3	6.06
#2	24	20	4	3	3	3.06
#3	12	20	4	3	3	1.56
#4	8	20	4	3	3	1.06
#5	6	20	4	3	3	0.81
#6	4	20	4	3	3	0.56

Experiments progressed from the longest HRT to the shortest HRT, and each of the HRTs was continuously run at least 5 cycles (i.e. replication 5 times). When conducting shorter HRT runs (HRT at 0.56, 0.81, and 1.06 days), a time interval of 2-5 days was given between the change of each HRT. If the organic contents were not

completely degraded under a shorter HRT, the microorganisms would continue to utilize the remaining nutrients. The given time interval was to minimize the effects of interactions among HRTs. HRT was calculated by the following equation:

$$HRT = V/Q \qquad (3.1)$$

where, V represents the working volume of reactor, which was 12 l in this study; and Q is the influent flowrate, which was calculated by: Q = 24/cycle time a day. The loading rate was calculated by:

$$Loading\ rate = Concentration\ of\ wastewater \times Q \qquad (3.2)$$

Where, the concentration of wastewater could be any of the parameters analyzed (TOC, BOD_5, COD, and SS).

3.3.3 Determination of optimal conditions of HRT and loading rate under suspended-growth condition

Factorial experiments were performed, with HRT and loading rate being two factors studied (Table 3.3). Three levels of HRT were used based on the previous experimental results (results from comparison of efficiencies of suspended growth and immobilized growth). Loading rates were mainly based on the different batches of wastewater

Table 3.3 Factors in the factorial experiments.

Factors		Aeration time (hr)		
		4	8	16
		HRT (day)		
		0.56 (θ_1)	1.06 (θ_2)	2.06 (θ_3)
Concentration of wastewater (TOC)	High (C_2)	$\theta_1 \times C_2$	$\theta_2 \times C_2$	$\theta_3 \times C_2$
	Low (C_1)	$\theta_1 \times C_1$	$\theta_2 \times C_1$	$\theta_3 \times C_1$

Table 3.4 Experimental schedules of factorial experiments.

Run	Reactor			
	1	2	3	4
1	$\theta_1 \times C_1$	$\theta_3 \times C_1$	$\theta_1 \times C_2$	$\theta_3 \times C_2$
2	$\theta_3 \times C_1$	$\theta_1 \times C_1$	$\theta_3 \times C_2$	$\theta_1 \times C_2$
3	$\theta_1 \times C_2$	$\theta_3 \times C_2$	$\theta_1 \times C_1$	$\theta_3 \times C_1$
4	$\theta_3 \times C_2$	$\theta_1 \times C_2$	$\theta_3 \times C_1$	$\theta_1 \times C_1$
5	$\theta_2 \times C_1$	$\theta_2 \times C_1$	$\theta_2 \times C_1$	$\theta_2 \times C_1$
6	$\theta_2 \times C_2$	$\theta_2 \times C_2$	$\theta_2 \times C_2$	$\theta_2 \times C_2$

- θ_1 = 0.56 days ; θ_2 = 1.06 days; θ_3 = 2.06 days;
- C_1 = Low loading rate; C_2 = High loading rate.

collected. As shown in Table 3.3, "Low" concentration (TOC) of wastewater was obtained by diluting raw brewery wastewater with tap water. "High" concentration of wastewater was the original brewery wastewater. Experimental schedules are given in Table 3.4.

Start-up of the aerobic SBRs in this study was achieved by seeding the four reactors with equal volume of activated sludge acclimated by brewery wastewater. The operation of the SBR was the same as described in Section 3.3.1. A complete factorial experiment was replicated 4 times (4 replicates).

3.3.4 Track studies of pH, TOC, and dissolved oxygen vs aeration time in suspended-growth SBRs

In this study, four reactors acted as four replications for each run. Before starting sampling, the reactors were reseeded, and activated sludge was acclimated by growing in brewery wastewater for a week. The operation of SBR was primarily the same as in Section 3.3.1, except for oxic feeding. Sampling was started from the first minute of feeding, and the time interval was 30 minutes. The aeration setting was fixed, and it allowed a dissolved oxygen level of 2.5-3 mg/l. Dissolved oxygen concentration was read by directly put the DO probes into the reactors.

3.4 Sampling and analytical methods

Influent, effluent, and sludge samples were taken for each run, and analyzed for TOC, BOD_5, COD, total solids, suspended solids, volatile solids (VS), suspended volatile solids (VSS) according to the Standard Methods (APHA, 1995). Total Kjeldahl

28

nitrogen(TKN), ammonia nitrogen (NH$_3$-N), nitrate nitrogen (NO$_3$-N), and orthophosphorus (orthol-P) were determined periodically using a autoanalyzer (Technicon) (Appendix table A-10).

To study microbial populations under suspended and attached-growth conditions, samples were taken periodically to examine the quantitative change of the microbial population under a microscope, in order to estimate the dominant and sub-dominant microbes.

Table 3.5 Sampling and analytical schedules for comparison of growth types.

Growth type	TOC	COD	BOD$_5$	SS	VSS
Suspended	2 × 4*	2 × 3	2 × 2	2 × 4	2 × 4
Attached	2 × 4	2 × 3	2 × 2	2 × 4	2 × 4

*: Analytical replication × sampling time

3.5 Data analysis

Statistical analyses were conducted by using the SAS software package (Anon, 1988). Correlation analysis was used to determine significance of relationships between variables (using the Pearson product-moment correlation, SAS procedure CORR). Statistical comparison of the results from suspended-growth and attached growth was performed by analysis of variance (ANOVA) and t-test (TTEST) was applied to detect the significance of differences between compared means.

The response surface regression (RSREG) procedure was employed. The RSREG procedure fits the parameters of a complete quadratic response surface and then

determine critical values to optimize the response with respect to the factors in the model. Many experiments were conducted to discover which factor values optimized a response. If a factor variable is measured at three or more values, a quadratic response surface can be estimated by least-square regression. The predicted optimal value can be found from the estimated surface if the surface is shaped appropriately. As mentioned in Section 3.3.3, two factors were selected and each of them had three (for HRT) or more (for loading rate) levels.

Unless stated otherwise, the level of significance used throughout these studies was set at $p < 0.05$, and the results of data analysis of means were reported as Mean ± SD.

Results and Discussion

4.1 Characteristics of brewery wastewater

Characteristics of the wastewater used in the experiments are summarized in Table 4.1. The wastewater, which was collected from a local brewery, was continuously discharged from various sources within the plant. The composition of the wastewater was typical of brewery wastewater which contained spent-grain, dead (or small amounts of live) yeast, spilled beer from fillers and packaging, and washing wastewater. The amount of wastewater discharged changed both diurnally and seasonally (Figure 1.1). This indicated that there were wide variations in the strength of the wastewater discharged, as shown in Table 4.1. These variations would undoubtedly affect the performance of treatment systems.

Table 4.1 Characteristics of brewery wastewater.

Parameter	Range of concentration
TOC	677-1720 ppm
BOD_5	671-4200 mg/l
COD	1038-4524 mg/l
TSS	450-1044 mg/l
TP	6.3-56 mg/l
Orthol-P	2-60 mg/l
TKN	28-343 mg/l
NH_3-N	0-4 mg/l
pH	6.1-9.5

Even though the inorganic nitrogen-nutrients were very low in the brewery wastewater (NH_3-N being around 0-4 mg/l), the nitrogen-nutrient was not a limiting nutrient, because the average TKN of mixed wastewater was about 343 mg/l. This average of TKN was higher than that of the supernatant wastewater (after solids settlement), which was about 34 mg/l. This is due to the spent grain and dead (or small amount of alive) yeast from fermentation contained in the solids contents of the wastewater. This suggested that most of the nitrogen-nutrient was in organic forms. Orthol-phosphate and total phosphate were sufficient for microbial growth. It has been reported that a suitable ratio of BOD:N:P is 160:4:1 for aerobic brewery wastewater treatment (Smith, 1986). Obviously, this ratio was attained in our experiments, and it was not necessary to add nitrogen-nutrients or phosphorus-nutrients to the reactors.

4.2 The start-up of SBRs

During the reactor start-up, the deterioration of biomass was observed. It was caused by fed with influent containing wastewater with low levels of organic content (BOD_5 being about 864mg/l) from the washing process. When these conditions pertained, sludge settling was poor and effluent suspended solids removal was lower than 75%. This suggests that the reactors would need to be re-seeded with fresh activated sludge in order to promote the recovery of biomass in the reactors. This would in turn affect the treatment efficiency of the SBR.

The correlation coefficients for the variables, which includes the effect of mixer, mixed liquor suspended solids (MLSS), and activated sludge concentration, clearly

indicate the importance of MLSS and activated sludge concentration for the performance of the reactors. As shown in Table 4.2, the correlation coefficient of MLSS and activated sludge concentration to BOD_5 removal was 0.887 and 0.853, respectively. It is understandable why the amount of MLSS and activated sludge were strongly correlated with BOD_5 removal and with reactor performance. Since SBR start-up is the process of biomass acclimating and accumulating, low concentrations of activated sludge and MLSS would lead to the biomass being washed out. As a result of insufficient biomass inside the reactor, the decomposition of organic compounds would not be achieved efficiently and therefore reactor performance would be poor.

Table 4.2. Correlation coefficients between studied variables (the upper value is the correlation coefficient, and the lower symbol represents the level of significance).

Variable	Mixing	MLSS	Act.sludge Concen.	BOD_5
MLSS	0.000 n.s.			
Act.sludge	0.000 n.s.	0.958 ***		
BOD_5	0.125 n.s.	-0.774 ***	-0.834 ***	
Removal	-0.053 n.s.	0.887 ***	0.853 ***	-0.768 ***

***$p < 0.001$, n.s. = not significant ($p > 0.05$).

The results from correlation analysis revealed no correlation between the function of the mixer and reactor performance; the correlation coefficient of the mixing effect and

BOD_5 removal was −0.053. This suggests that the reactors could be run without using a mixer. In order to verify this suggestion, an additional t-test was conducted to compare the mean values of BOD_5 removal from a reactor with a mixer and from a reactor without a mixer. As expected, results of the t-test demonstrated that the difference in performance between the reactor with mixer and the reactor without mixer was not significant ($F = 1.17$, $p = 0.794$). It seemed that sufficient mixing could be achieved inside the reactors by the force of aeration.

A higher air flow rate (> 4 l/min) was set after the mixer was removed. This resulted in the disruption of sludge particles. A large amount of sludge in a fine particle form was suspended during the aeration and failed to settle in the serttling stage. Under these conditions, the effluent SS removal was about zero. The reactors were then re-seeded with acclimated sludge and air flow rate was reduced to 2.5-3 l/min. This aeration rate was then retained throughout the subsequent experiments.

After the sludge had built-up in the reactors, the operation of SBRs attained a quasi-steady state. This was distinguished by a consistent concentration of TOC in the effluent and the visual observation of healthy sludge conditions in the reactors.

4.3 Comparison of the performance of suspended-growth and attached-growth aerobic SBRs

4.3.1 The effects of HRT and loading rate under two types of growth conditions

Figure 4.1 shows the percentage removal of TOC influent brewery wastewater for different loading rates and under conventional suspended-growth and attached-growth biofilm conditions.
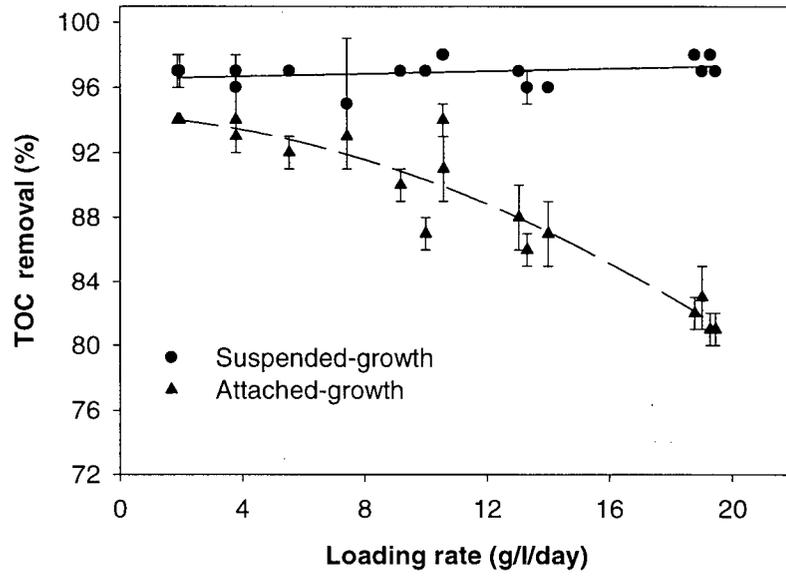
Figure 4.1 TOC removal vs loading rate.
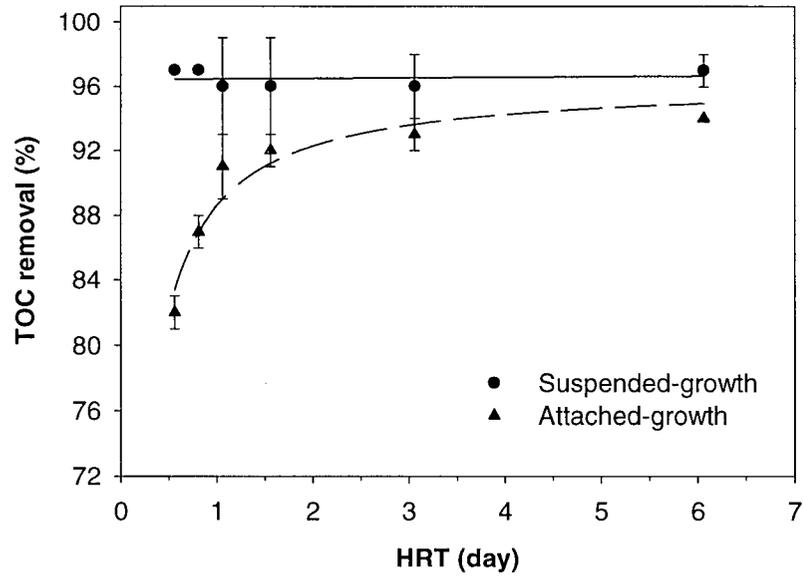
Figure 4.2 TOC removal vs HRT.

As shown in Figure 4.1, the TOC removal trends in suspended-growth conditions differed from that in attached-growth conditions. When the loading rate was about or below 10 g/l/day, the overall percentage of TOC removal attained was over 90% under both growth conditions. The TOC percentage removal decreased in the attached-growth reactors as the loading rate increased. However, little change was detected in suspended-growth reactors in term of TOC removal at different loading rates.

The effects of HRT on the performance of the reactors under different growth conditions are shown in Figure 4.2. The TOC removal trends as a function of HRT in suspended-growth were also different from that in attached-growth reactors. The effects of HRT on TOC removal in suspended-growth conditions were not as significant as they were under attached-growth conditions. Under attached-growth conditions, the TOC percentage removal increased with increasing HRT until the latter reached a certain point (within 2-3 days of HRT as shown in Figure 4.2). After that point, TOC removal levelled out with increasing HRT. The experimental results of the effects of HRT on the treatment efficiencies are summarized in Table 4.3.

Parallel to the TOC analysis, BOD_5 and COD analyses were conducted for these same samplings. Similar trends were found for the effects of HRT and loading rate on BOD_5 and COD percentage removals.

Figure 4.3 displays BOD_5 removal as a function of loading rate, and Figure 4.4 plots COD removal vs loading rate. Comparison of these two figures with Figure 4.1 reveals a general similarity. TOC, BOD_5 and COD removal decreased as the loading rate
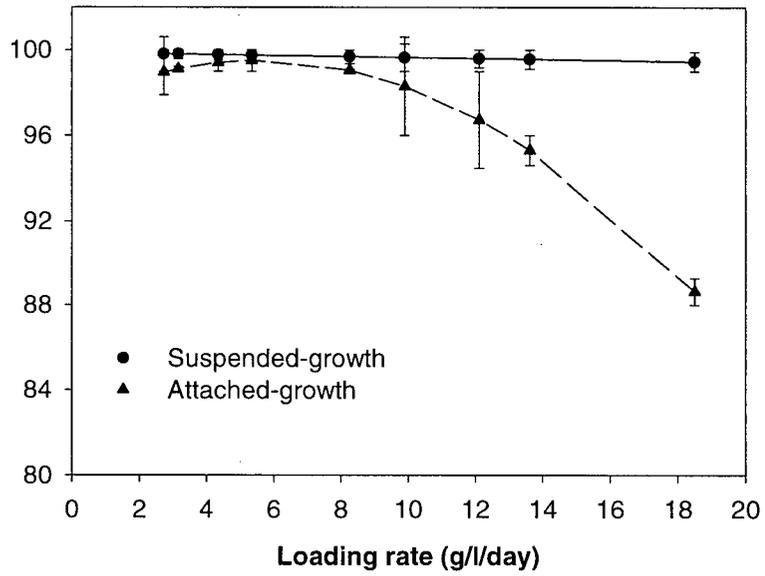
Figure 4.3 BOD$_5$ removal vs loading rate.
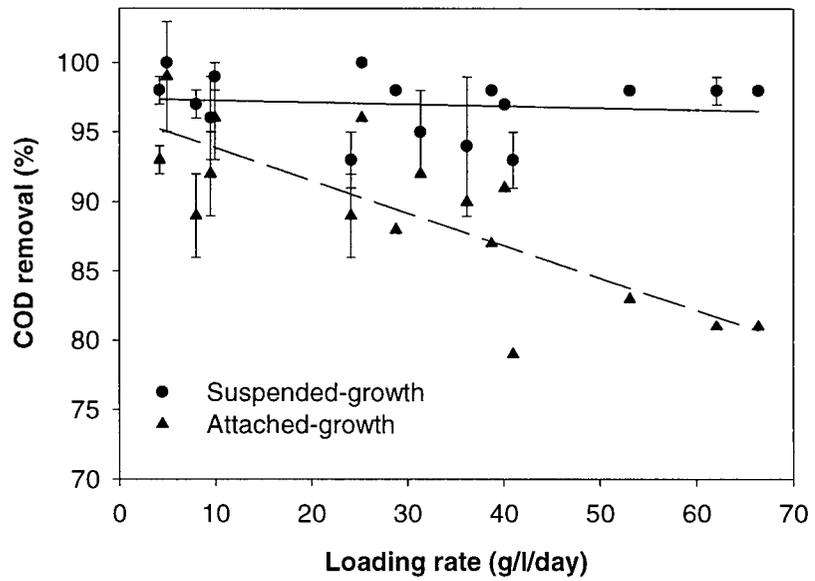
Figure 4.4 COD removal vs loading rate.

Table 4.3 Effects of HRT on the treatment efficiencies of aerobic SBR under

suspended-growth and attached-growth conditions.

HRT (day)	Factor	Influent (mg/l)	Sampling No.	Effluent (mg/l)		Percentage removal	
				Suspended	Attached	Suspended	Attached
0.56	TOC	896.8±14.13	16	23.1±4.6	164.1±14.5	0.97±0.00	0.82±0.01
	COD	2837±317.85	12	61.53±17.81	551.4±55.01	0.98±0.01	0.81±0.01
	BOD$_5$	929.5±88.39	8	10.92±1.41	98.43±8.84	0.99±0.00	0.89±0.02
	SS	585±183.85	16	10.78±3.55	140.28±21.66	0.98±0.01	0.74±0.09
0.81	TOC	852.3±120.00	16	27.57±6.17	108.61±18.60	0.97±0.00	0.87±0.01
	COD	2449.3±439.83	12	66.3±20.94	273.3±70.13	0.96±0.02	0.84±0.04
	BOD$_5$	1018.0±490.73	8	36.30±33.67	43.81±26.69	0.97±0.02	0.96±0.01
	SS	597.5±17.68	16	9.91±3.83	100.7±25.23	0.98±0.01	0.83±0.04
1.06	TOC	905±62.00	16	32.55±31.07	79.17±18.81	0.96±0.03	0.91±0.02
	COD	2853.3±661.99	12	89.43±81.08	223.5±124.9	0.97±0.03	0.93±0.03
	BOD$_5$	1548.0±483.66	8	20.30±28.34	43.12±12.29	0.99±0.01	0.97±0.01
	SS	706.3±249.04	16	25.03±37.95	128.3±58.83	0.97±0.04	0.80±0.11
1.56	TOC	904.3±122.83	16	37.12±28.77	63.46±13.58	0.96±0.03	0.920±.01
	COD	2962.3±1841.9	12	142.2±112.0	236.9±135.1	0.94±0.03	0.89±0.03
	BOD$_5$	1327.5±354.97	8	0.53±1.13	12.07±5.17	1.00±0.00	0.99±0.01
	SS	638.7±292.82	16	18.88±21.48	72.63±42.78	0.97±0.02	0.87±0.07
3.06	TOC	965.5±0.577	16	34.81±18.34	63.3±8.81	0.96±0.02	0.93±0.01
	COD	2493.7±64.66	12	44.47±60.21	128.21±61.16	0.98±0.02	0.95±0.03
	BOD$_5$	1237.5±180.31	8	3.34±3.21	12.83±4.72	1.00±0.00	0.99±0.00
	SS	1044	16	41.44±38.36	54.25±39.8	0.96±0.04	0.95±0.05
6.06	TOC	966.5±20.23	16	27.23±7.90	58.09±3.14	0.970.0±1	0.94±0.00
	COD	2359.3±222.86	12	21.18±25.91	104.3±51.16	0.99±0.01	0.95±0.03
	BOD$_5$	1489.0±154.15	8	10.33±7.67	10.27±7.88	0.99±0.01	0.99±0.00
	SS	1044	16	30.81±12.08	33.69±14.48	0.97±0.01	0.97±0.01
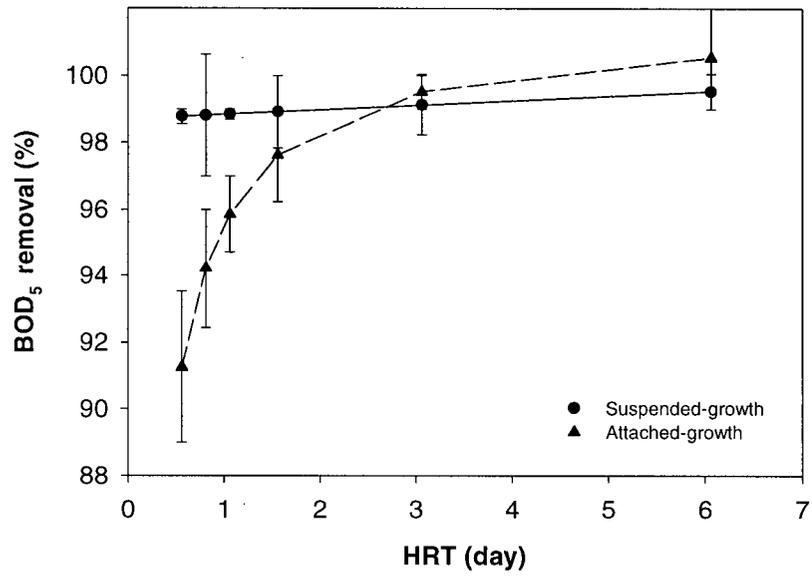
Figure 4.5 BOD$_5$ removal vs HRT.
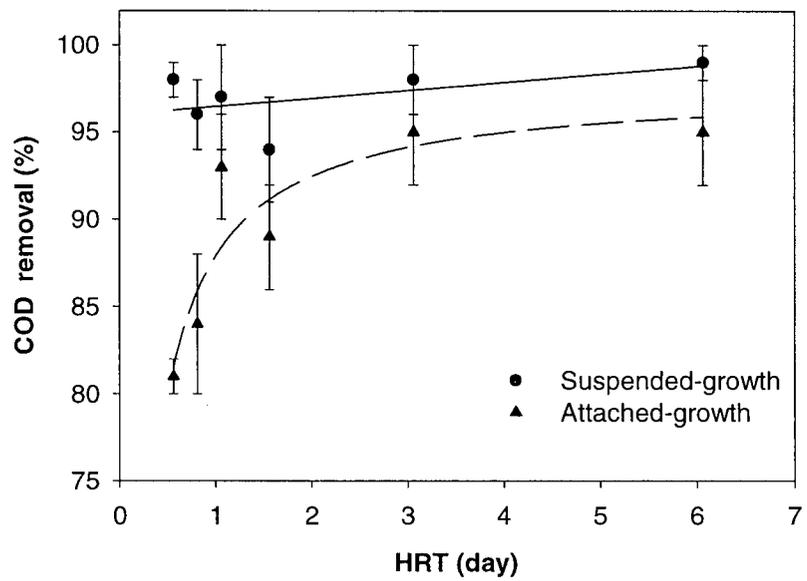
Figure 4.6 COD removal vs HRT.

increased in the attached-growth reactors, while in the suspended-growth reactor, the changes of loading rate were not reflected in changes in TOC, BOD_5, and COD removal. COD removal was more sensitive to the effects of loading rate in both types of growth conditions than were BOD_5 and TOC removal. A linear decrease of COD removal was seen in attached-growth SBRs as loading rate increased (Figure 4.4).

Figure 4.5 and Figure 4.6 show BOD_5 and COD removal as a function of HRT. As HRT increased, the COD removal in the attached-growth SBRs increased. However, it could never reach the same level as that in the suspended-growth SBRs. On the other hand, it appears possible for BOD_5 removal to be increased to the same levels for both growth regimes if the HRT was longer than 3 days.

Based on the above results, it was inferred that the effect of HRT was more pronounced than that of loading rate on the performance of an aerobic SBR. This could be attributed to the fact that loading rate is determined by either the wastewater concentration or the influent flow rate, and HRT is related to loading rate by the influent flow rate (see Equation 3.1 and Equation 3.2). This inference was proven by the following results of statistical analysis.

Table 4.4 contains the statistical results of HRT and loading rate effects on TOC percentage removal. Both HRT and loading rate had significant effects (at 90% confidence level). The effect of HRT was more sensitive to the growth regime than was the effect of changes in the loading rate.

The effects of both HRT and loading rate on suspended solids removal were generally similar to those of BOD_5, TOC, and COD. The percentage removal of

suspended solids decreased slightly as HRT increased in suspended-growth reactors (Figure 4.7). Increased HRT facilitated solid removal in the attached-growth reactors but longer HRT (particularly over 4 days) would not stimulate higher solid removal as shown in Figure 4.7. As would be expected, suspended solids removal decreased as loading rate increased in the attached-growth reactors, and it remained at nearly the same levels in the suspended-growth reactors (Figure 4.8).

Table 4.4. Summary of statistical analysis of HRT and loading rate effects on the performance of SBRs under different growth conditions.

Variable Effects (TOC)	Suspended-growth		Attached-growth	
	F value	p value	F value	p value
HRT	1.89	0.10*	239.4	0.000***
Loading rate	3.21	0.000***	3.14	0.001***

Level of significance is 0.1. *$p<0.1$, ***$p<0.001$.

It was known that the influent wastewater contained rather high levels of both organic carbon-nutrients and nitrogen-nutrients. Microbial activity degraded nutrients inside the reactors and available nutrients were exhausted as the HRT increased. The depletion of nutrients led to sludge bulking and poor sludge settling. Suspended solids removal therefore decreased, as shown in Figure 4.7. Experimental results also revealed that a portion of the suspended solids could be removed by attachment to the Ringlace in the attached-growth reactors.
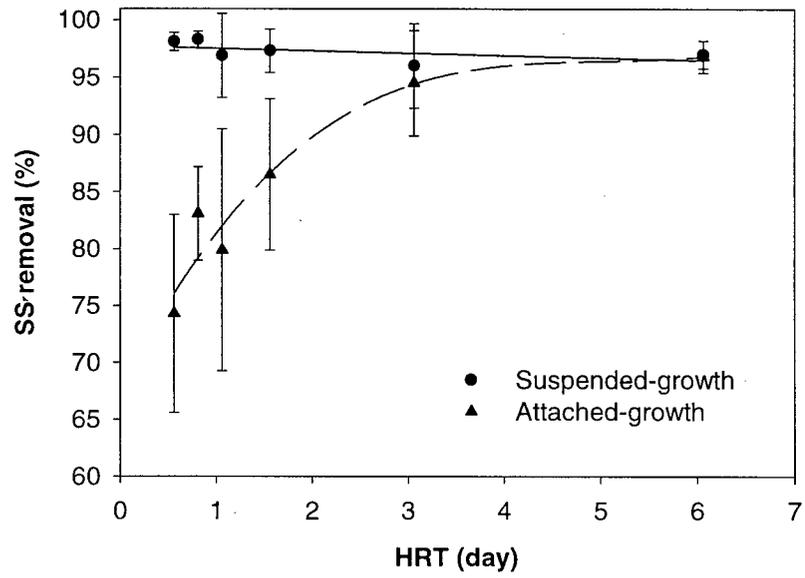
Figure 4.7 SS removal vs HRT.
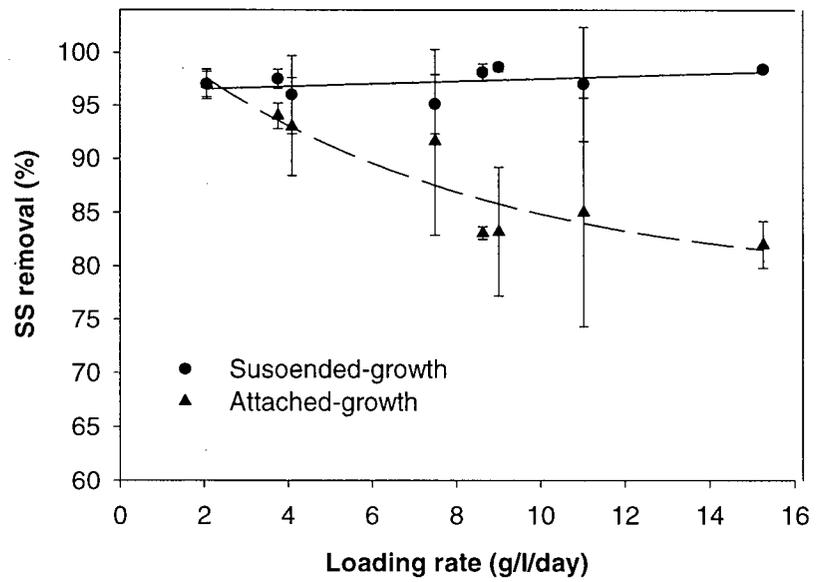
Figure 4.8 SS removal vs the loading rate.

42

4.3.2 Effects of growth types on the performance of aerobic SBRs

The results of these experimental studies have demonstrated that the aerobic SBR is able to achieve a high level reduction in the levels of polluting components in brewery wastewater. The removal of TOC, BOD_5, and COD was over 90% in both the suspended-growth reactors and the attached-growth reactors (as shown in Table 4.5). Statistical analysis demonstrated that the effect of the two growth type regimes on the performance of SBR was significantly different. Comparison of the treatment efficiencies revealed that the performance of the suspended-growth SBRs was significantly higher than that of the attached-growth SBRs (Table 4.6).

In general, the brewery wastewater used in this study contained soluble, colloidal, and settleable organic materials. These organic materials can be removed by direct assimilation, adsorption, flocculation and coagulation, and settling. Thus there was rapid removal of the organic materials and a sharp drop of concentrations of TOC, BOD_5 and COD from the wastewater within reactors.

Low molecular weight soluble, organic compounds can be assimilated directly by microbial cells using active, facilitated, and passive transport mechanisms. Large colloidal particles are also susceptible to removal by surface phenomena. Particles removed by flocculation and coagulation are expected to include cells, cell fragments, viruses, lipid micelles and other organic debris. The rapid removal of soluble materials arose for several reasons. Higher rates of molecular diffusion and enzyme specificity at

Table 4.5. The summary of the effects of growth types on BOD_5, COD, and TOC removal.

| | Percentage removal | | Influent concentration | Effluent concentration | |
	Susupended-growth.	Attached-growth		Suspended-growth	Attached-growth
TOC	0.97±0.02	0.90±0.05	677-984 ppm	30.4	89.46
BOD_5	0.99±0.01	0.97±0.04	671-1890 mg/l	13.62	36.75
COD	0.97±0.03	0.9±0.06	1038-4709 mg/l	70.85	252.94
SS	0.97±0.02	0.86±0.1	450-1044 mg/l	22.81	88.30

Table 4.6. Comparisons for the means of TOC, BOD_5, COD, and SS from suspended-growth and attached-growth reactors (t-test).

	df	F value	p value
TOC	(95, 95)	5.19	0.0000
BOD_5	(47,47)	6.64	0.0000
COD	(71,71)	4.86	0.0000
Suspended Solids	(95,95)	17.81	0.0000

least partly resulted in the rapid removal. Molecules taken into the cells may then be channeled into both anabolic and catabolic pathways, producing new cellular materials while providing energy for synthesis and other microbial activity. Macromolecules too large to be directly assimilated are likely to be removed from solution prior to enzymatic attack and further metabolism by adsorption phenomena on the surface in the pore spaces of biological flocs (Yu et al., 1997).

The significant difference in the results between the two growth types may be related to the oxygen transfer efficiency and the configuration differences between the reactors. Yu et al. (1997), using SBRs to treat brewery wastewater, observed that the microorganism in SBRs utilized the accumulated substrates at a very high rate when air was added. Under suspended-growth conditions, the oxygen was efficiently used resulting in healthy microbial growth. Under attached-growth conditions, the metabolic efficiency would be lower because the microbes attached to the Ringlace (the diameter of attached sludge floc was about 1.5-3 cm), and the inner layer could not access sufficient oxygen.

In suspended-growth SBRs, sludge particles had an equal chance to come into contact with the nutrients, and substrates could therefore be utilized at maximum efficiency given sufficient aeration and mixing. While the attached-growth systems involved solid supports media (Ringlace) on which biomass developed, a large specific surface area of Ringlace would increase the amount of attached biomass. The mixed liquor contacted the outermost surface of the biomass layer. The nutrients required for life inside that layer were obtained by diffusion into the surface from the mixing liquor

flowing past (Barnes et al., 1983). In addition, mixing conditions in the attached-growth reactors were not as good as in the suspended-growth reactors, due to obstruction by the biomass attached to the Ringlace in the attached-growth SBRs. The utilization of nutrients was therefore less efficient in the attached-growth reactors than in the suspended-growth reactors.

The experimental results from this study show that only slight changes occurred in the suspended-growth SBRs with variations in HRT and loading rate. This is in accordance with the properties of SBRs. The SBR provides for a diverse array of operating conditions and selective pressures and can thus become a versatile tool for the enrichment of specific consortia and induction of a desired metabolic pathway. By adding the system's own periodicity or forcing function, the potentially negative impact of those forcing functions associated with variations in composition and concentration of wastewater, operation time, and other factors can be mitigated (Irvine et al., 1997).

4.3.3 Observation of microorganisms by microscope

The observations of microbial populations by microscopy demonstrated that the populations consisted mainly of healthy floc-forming bacteria under suspended-growth conditions (Figure 4.9), while under attached-growth conditions, filamentous microbial forms were dominant in the reactors (figure 4.10). The luxuriant growth of filamentous organisms was associated with the notorious condition known as "bulking" in which the sludge is difficult to separate from the treatment effluent by settlement. These phenomena were also noted when the reactors were loaded with highly concentrated wastewater or
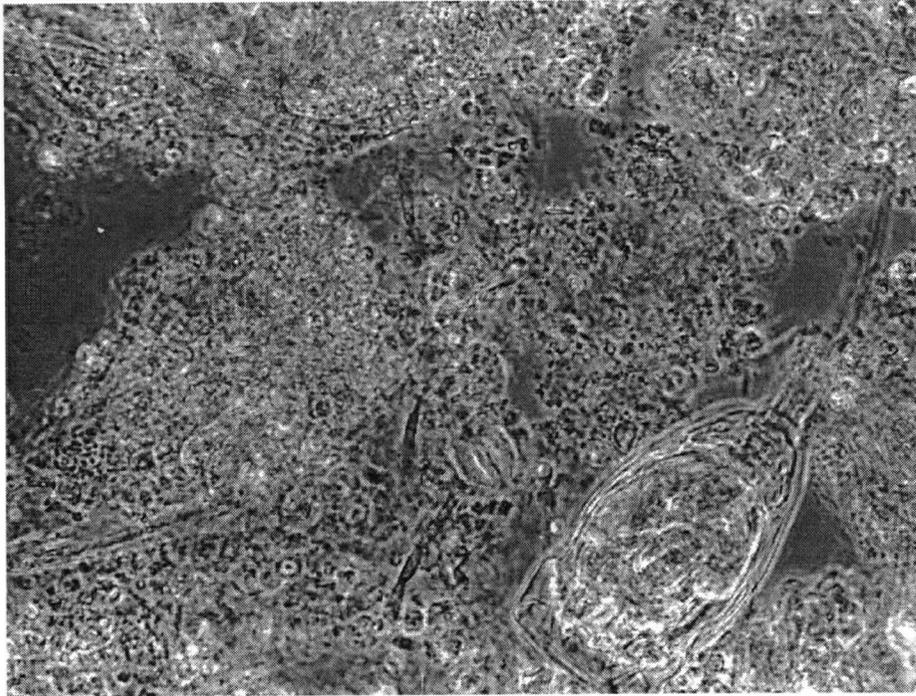
Figure 4.9 The photograph of microbe taken from

suspended-growth reactor (100X).
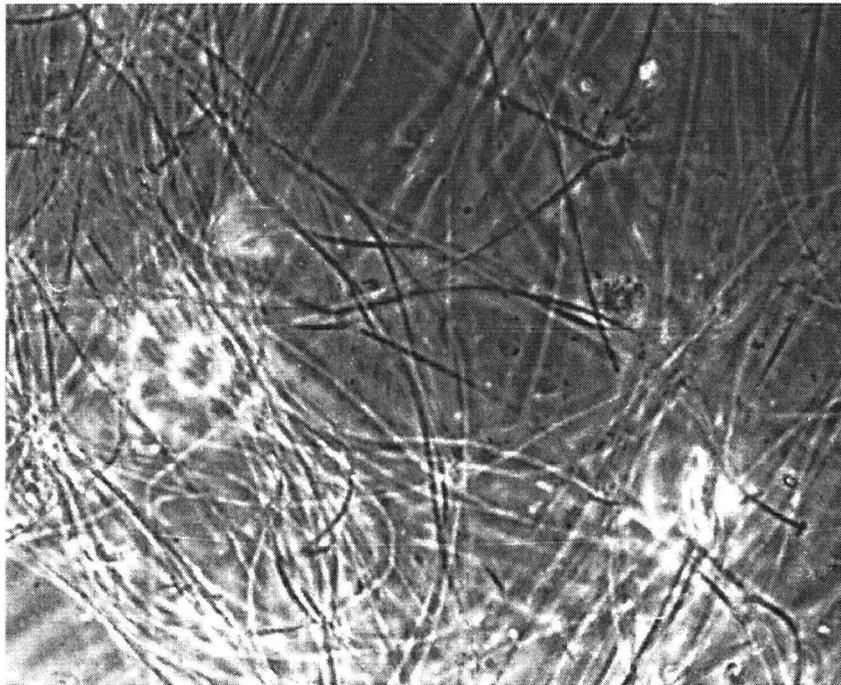
Figure 4.10 The photograph of microbe taken from attached-growth reactor (100X).

run at a shorter HRT under attached-growth conditions. As seen in Table 4.3, the SS removal was about 74% at 0.56 days of HRT.

Bacteria, algae, ciliated protozoa/rotifer, and worms that were commonly found in domestic wastewater treatment processes were observed in both growth regimes. An effort was made to estimate the quantitative changes in the microbial species. Even though the results failed to be statistically convincing, these observations will be useful for further studies. The quantitative changes of certain microbial species were mainly determined by the environmental conditions such as nutrients, pH, temperature, light, and oxygen. It was noted that there were generally more worms growing in the attached-growth reactors than in the suspended-growth reactors. However, excessive amounts of roundworms were found in the suspended-growth reactors when they were run at a longer HRT and over aeration was used. It was suspected that the depletion of nutrients was one of the reasons for the growth of the worms. In addition, one experiment has found that fluctuations in dissolved oxygen levels appeared to affect worm growth; an increase in the dissolved oxygen concentration stimulated the propagation of worms (Setter, 1995). Red color roundworms were mostly located on the upper part of reactors where the oxygen concentration is higher than in the lower levels.

Some species of rotifer grew in the suspended-growth reactors. Rotifers are very effective in consuming dispersed and bacteria in small flocs. Their presence in the effluent may indicate a highly efficient aerobic biological purification process.

4.3.4 Correlation among TOC, BOD$_5$, and COD

Establishment of the constant relationship among the various measures of organic content depends primarily on the nature of the wastewater and its source. Table 4.7 listed the ratios of TOC, BOD$_5$, and COD. The ratios of influent were calculated based on the average values of TOC, BOD$_5$, and COD from the measures of raw wastewater collected, and the ratios of effluent were calculated based on the average values of TOC, BOD$_5$, and COD from the treated effluent. BOD$_5$/COD is a good indicator of biodegradability of wastewater. The average ratio of BOD$_5$/COD in the influent was 0.58, which indicated that 58% of materials the influent wastewater were biodegradable. After treated by aerobic SBRs, the average ratio of BOD$_5$/COD in the effluent dropped to 0.15. This value indicated that there was little biodegradable matter left. The accumulation of microbial metabolic products and non-biodegradable materials decreased the BOD$_5$ as well as the BOD$_5$/COD ratio. It should be noted that the ratios of effluent vary considerably with the degree of treatment that the wastewater has undergone, therefore, the ratios listed here were relative values, and should not be used else where.

Table 4.7 The ratios of TOC, BOD$_5$, and COD.

	TOC/BOD$_5$	TOC/COD	BOD$_5$/COD
Raw wastewater	0.78	0.44	0.58
Effluent	4.86	0.48	0.15

Since the measurements of TOC were simple and had a higher precision than the BOD$_5$ and COD measurements, only the TOC of the samples was analyzed in the following experiments, Estimation of BOD$_5$ and COD were made based on the initially determined ratios of TOC/BOD$_5$ and TOC/COD.

4.4 Optimal conditions of HRT and loading rate in suspended-growth SBR

4.4.1 The analysis of variances among four reactors

Four suspended-growth reactors were run under identical conditions for about 10 days before factorial experiments were started. It is supposed that the sludge conditions in these four reactors were the same. The pH fluctuated between 6-8 and was monitored periodically.

The correlation coefficients of the four reactors are listed in Table 4.8. The purpose of this correlation analysis was to analyze the consistency of the measured values from the four different reactors for the same variables. The null hypothesis was that there is no difference between the four reactors ($\alpha = 0.05$). The results showed that the highest correlation coefficient between reactors was 0.76911, which was between reactor 2 and reactor 4, with $p = 0.0001$. The lowest correlation coefficient was 0.30698, which was between reactor 3 and reactor 1, and its probability was 0.0338. Thus, it was concluded that there was no significant difference among the four reactors (Table 4.8).

Table 4.8 Pearson correlation coefficient of the four reactors (the upper value is the correlation coefficient, and the low symbol represents the level of significance).

	Reactor 1	Reactor 2	Reactor 3
Reactor 2	0.515 ***		
Reactor 3	0.307 *	0.677 ***	
Reactor 4	0.740 ***	0.769 ***	0.613 ***

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Table 4.9 The ANOVA for the four reactors

Sources of variance	Reactors
Degree of freedom	3
Sum of Squares	0.01483996
Mean Square	0.00494665
F value	1.52
$Pr > F$	0.2105

Experiments were performed in these four reactors, and each of the pair-wise combination of two factors was assigned to one of the reactors as randomly as possible. The ANOVA was performed to analyze the variance of the reactors, and results are listed in Table 4.9. With $F = 1.52$, and the significance probability value associated with this F value 0.2105, the conclusion drawn above was confirmed. That is, the variances among the reactors were not statistically significant. Based on the above conclusion, the variations among the four reactors could be ignored. The results from the four reactors could be treated as replicates under their corresponding experimental conditions according to the following analysis.

4.4.2 Correlation between variables

The effects of different variables on the values of TOC removal were tested, and the results were described in the following Table 4.10. ANOVA was used to determine the significance of these variables (Table 4.11). As shown in the results (Table 4.10 and 4.11), samplings were not correlated with TOC removal, nor was the variance in samplings significant ($\alpha = 0.05$). Even though the Pearson correlation analysis showed that the correlation coefficient between replication and TOC removal was not significant, ANOVA indicated a slight significance in variances of replications at ($p = 0.102$). TOC removal had a positive correlation with HRT, but had a negative correlation with loading rate and the concentration of influent. Thus, particular attention was paid to the relative dominance of HRT and loading rate effects. The HRT and loading rate were strongly correlated (r = –0.811). The relationship between HRT and loading rate can be described by Equation 3.2.

Table 4.10 Correlation coefficient (upper row) and level of significance (lower row) between variables under suspended-growth conditions

Variable	Replication	HRT	Loading	Concentration	Sample
HRT	0.000				
	n.s.				
Loading	-0.003	-0.811			
	n.s.	***			
Concen	0.0195	0.053	0.427		
	n.s.	n.s.	***		
Sample	0.000	0.000	0.000	0.000	
	n.s.	n.s.	n.s.	n.s.	
Removal	0.0608	0.354	-0.578	-0.224	0.016
	n.s.	***	***	**	n.s.

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and n.s. = not significant.

Table 4.11 Summary of ANOVA in the factorial experiments

Variable	DF	ANOVA SS	F value
Replication	3	0.0202	2.10^*
HRT	3	0.3226	100.38^{***}
Loading rate	23	0.437	16.9^{***}
Concentration	13	0.2218	7.51^{***}
Sample	1	0.0001	$0.05^{n.s.}$

$* p < 0.1$, $*** p < 0.001$, n.s. = not significant.

4.4.3. Response surface analysis for the effects of HRT and loading rate on TOC removal

Table 4.12 shows the response surface analysis of TOC removal vs loading rate and HRT. The response variable (dependent variable) was TOC removal; HRT and loading rate were treated as controlled variables. The mean value of response was 0.91 (TOC removal), and R-square was about 0.52 as displayed in Table 4.12. The relatively low value of the coefficient of variation (CV = 4.38) indicated good precision and great reliability of the experiments carried out. An optimum point (maximum point as well) of TOC removal was obtained by this response surface analysis, which was around 97% of TOC removal under HRT = 1.44 days with 3122 ppm/day of TOC loading rate.

Table 4.12 Response surface analysis of TOC for the effects of HRT

and loading rate.

(a) Parameters of TOC removal response surface

Variable	TOC percentage removal
Response mean	0.913812
Root MSE	0.040004
R-Square	0.5247
Coef. of Variation	4.3777

(b) Critical Value

Factor	Coded	Uncoded
HRT (day)	0.170594	1.437945
LOADING (ppm/day)	-1.101631	3121.550113
Predicted value at stationary point*		0.979589

(c) Eigenvectors

Eigenvalues	HRT	LOADING
-0.010578	-0.043942	0.999034
-0.077435	0.999034	0.043942

* Stationary point is a maximum

The application of response surface methodology yielded the following regression equation which is an empirical relationship between the dependent variable and the test variables in coded unit*:

$$Y = 0.965 + 0.020\,X_1 - 0.023\,X_2 - 0.077\,X_1^2 - 0.006\,X_1X_2 - 0.011X_2^2 \tag{4.1}$$

Where Y is the response, (i.e. the TOC removal), and X_1 and X_2 are the coded values of the test variable HRT and loading rate, respectively. TOC removal with different HRT and loading rates can be predicted by using this regression equation.

The significance of each coefficient, which are listed in Table 4.13, was determined by t-test. The larger the magnitude of t-value and smaller the p-value, the more significant is the corresponding coefficient. This implies that the quadratic main effect of HRT ($p < 0.0002$) on the TOC removal was more significant than its first order effect. Both first order and quadratic effects of HRT were highly significant ($p < 0.05$). The loading rate had less effects on the TOC removal than did HRT, and the interaction between HRT and loading rate did not have a significant influence on the TOC removal ($p < 0.90$). This result was consistent with that given by the eigenvectors** (Table 4.12). As shown in Table 4.12, the HRT has higher eigenvectors than does loading rate.

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*Coded units/coded data: are standardized data. In this case, $HRT_{coded} = (HRT_{uncoded} - 1.31)/0.75$; TOC loading $rate_{coded} = (TOC$ loading $rate_{uncoded} - 20719)/15974$.
**Eigenvalue/eivectors: are from the matrix of quadratic parameter estimates, which determine the curvature of the response surface.

A 3-dimensional graph was constructed to show the relationship between TOC removal vs HRT and loading rate. This graph was based on the regression data, with HRT on the abscissa, loading rate on the ordinate, and TOC removal perpendicular to the plane (Figure 4.11). This is a quadratically smoothed surface. The relatively flat response surface along the axis of ordinate, which represents the loading rate, indicates that a relatively wide variation can be tolerated without TOC removal being seriously affected. The very pointed surface seen along the axis of abscissa, which represents HRT, suggests that the TOC removal would be sensitive to changes of HRT.

Table 4.13 The significance of regression coefficient (TOC).

Model term	Coefficient (coded)	Standard Error	t-value	p-value
Intercept	0.965	0.140	4.933	0.0000
X1	0.020	0.144	2.765	0.0063
X2	-0.023	0.000	0.127	0.8988
X1^2	-0.077	0.036	-3.799	0.0002
X2^2	-0.011	0.000	-0.393	0.6950
X1X2	-0.006	0.000	-0.130	0.8967
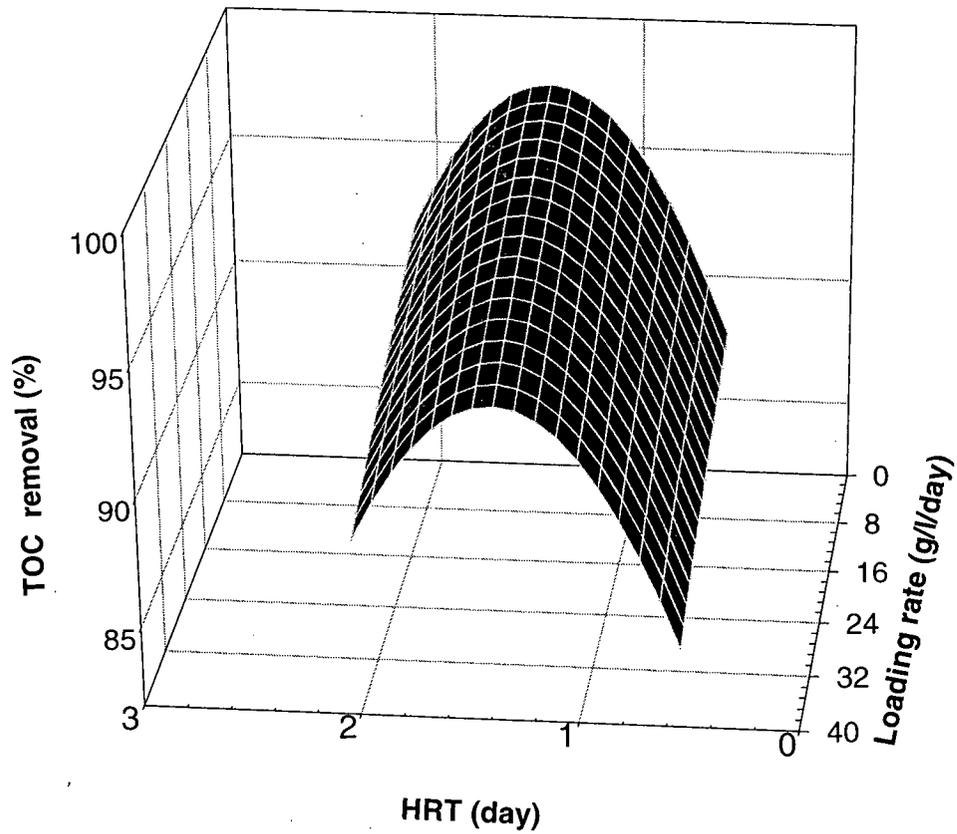
Figure 4.11 Response surface of TOC removal vs HRT vs loading rate vs in suspended-growth reactors.

4.4.4 Response surface for the effects of HRT and loading rate on SS removal

The HRT and loading rate effects on the suspended solids removal in aerobic SBRs was also analyzed using the response surface analysis. The results are shown in Table 4.14. In this analysis, SS removal was the response variable, which is a function of HRT and loading rate. The mean of SS removal on the response surface was 91.6%, and R-square was 0.389. With the coefficient of variation being about 5.16, the experiments were considered to be reliable. To achieve the maximum percentage removal of SS in this response regression, the predicted critical values of HRT and loading rate are 1.44 days and 13,838 mg/l/day SS, respectively.

The regression equation of this response surface is generated as the following Equation 4.2 which is in coded units*:

$$Y = 0.996 + 0.017\, X_1 + 0.046\, X_2 - 0.090\, X_1^2 - 0.092\, X_1X_2 - 0.142\, X_2^2 \qquad (4.2)$$

Where Y is the response (i.e. the SS removal), and X_1 and X_2 are the coded values of the test variable HRT and loading rate, respectively. The SS removal with different HRTs and loading rates can be explained using this regression equation.

The significance of each coefficient of variables to SS removal was determined by *t*-tests, which are listed in Table 4.15. Both the first order main effect and the quadratic main effect of HRT are highly significant ($p < 0.05$). This is in good accordance with the

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*Coded units/coded data: are standardized data. In this case, $HRT_{coded} = (HRT_{uncoded} - 1.31)/0.75$; SS loading $rate_{coded} = (SS$ loading $rate_{uncoded} - 12559)/8174$.

Table 4.14 Response surface results of SS removal for the effects of HRT

and loading rate.

(a) Parameters of SS removal response surface	
Variable	SS percentage removal
Response Mean	0.916292
Root MSE	0.047314
R-Square	0.3890
Coef. of Variation	5.1637

(b) Critical value		
Factor	Coded	Uncoded
HRT (day)	0.014917	1.321188
LOADING (mg/l/day)	0.156940	13838
Predicted value at stationary point*	1.00000	

(c) Eigenvectors		
Eigenvalues	HRT	LOADING
-0.063279	0.863852	-0.503746
-0.169217	0.503746	0.863852

* Stationary point is a maximum.

results from section 4.3.2. However, the SS removal was significantly affected by the loading rate, and the influence of the interaction between HRT and loading rate was not statistically significant ($\alpha = 0.05$). The eigenvalues shows that the loading rate had a slightly stronger effect on SS removal than did the HRT (Table 4.14).

Table 4.15 The regression coefficient of response surface of SS removal vs HRT and loading rate.

Model term	Coefficient (coded)	Standard error	t-value	p-value
Intercept	0.996	0.248	0.136	0.8920
X1	0.017	0.165	3.840	0.0002
X2	0.046	0.000	2.863	0.0052
$X1^2$	-0.090	0.035	-4.646	0.0000
$X2^2$	-0.092	0.000	-1.821	0.0719
X1X2	-0.142	0.000	-2.878	0.0050

Figure 4.12 is a 3-dimensional graph of the response surface showing the relationships between SS removal, HRT, and loading rate. Here, loading rate is the abscissa, HRT is on the ordinate, and response variable (SS removal is perpendicular to the plane (HRT and loading rate). This response surface is a rather pointed surface, especially in the direction of the loading rate, which suggests that the SS removal is very sensitive to variations in the loading rate.
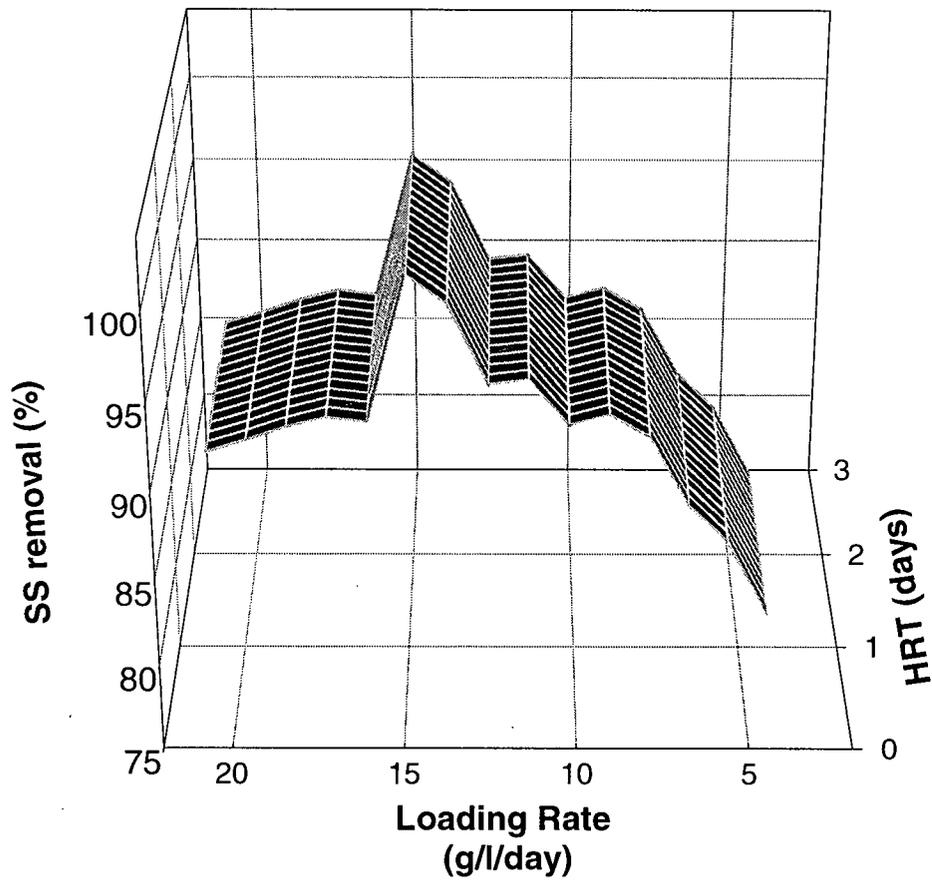
Figure 4.12 Response surface of suspended solids vs HRT vs loading rate in suspended-growth reactor.

Differences in the effects of HRT and loading rate on TOC removal and SS removal may be explained by the differences in the characteristics of the wastewater. As mentioned in Section 4.1, brewery wastewater contains a large amount of solids waste. These solids contain "non-volatile" materials, including spent grain. While these solids (except for yeast) contain no biomass, they do take up room in the reactor. Since only small molecules can be absorbed into microorganisms and afterwards digested, the large particles would be removed by surface phenomena, and the removal of solids by surface phenomena can be limited. As mentioned in Section 4.3.1, the effect of loading rate on the performance of suspended-growth SBR can be related to HRT, as well as to the concentration of the wastewater. Microbial growth would flourish with sufficient nutrients, although a higher loading rate could stop microbial growth due to the impact of shock and to a choking effect caused by the overloading of the solids. In our study, we observed that the increase in the concentration of influent (especially with higher solids concentrations) did result in a decrease in treatment efficiency.

In general, a longer HRT gave more time for microbial action to degrade the organic content of the wastewater. Treatment efficiencies therefore increased with an increasing HRT. This did not, however, imply that the longer HRT, the higher the efficiency. As shown in the results of Section 4.3.2, an optimal HRT does exist. Since the SBR is run in batch mode, the nutrients are fed to the reactors at the beginning of the sequencing cycle. As the reaction stage goes on, the nutrients will be exhausted, and the concentration of any toxic compounds will increase in the reactor with a longer HRT

(Schmit et al., 1994). Thus, a too-long HRT can result in a decrease in treatment efficiency.

4.5 Track studies of TOC, DO, and pH vs aeration time in suspended-growth SBRs

4.5.1 Variations among the reactors

The four reactors were run as replications for this continuous track study of TOC, dissolved oxygen, and pH. Figure 4.13 pictures the patterns of TOC degradation vs aeration time in the four reactors respectively. It was showed that reactors 1 and 4 were more similar than were the other two reactors. Reactor 2 may have had a higher degradation efficiency. It was demonstrated that the variation of the four reactors was statistically significant (Table 4.16).

To minimize the variance from the four reactors, the four reactors were grouped based on the results from statistical multiple comparison (Fisher's Least Significant Difference — LSD). In Table 4.17, the means with the same letter are not significantly different, which suggests that they can be grouped together. The results of LSD shows that reactor 2 was certainly in a different group than reactor 1 and 4, while reactor 3 could be grouped with reactor 2 or with reactors 1 and 4 (Table 4.17). Based on this result, reactor 2 was excluded in the final data analysis when averaging TOC concentration, and the average of the data were calculated only from reactors 1, 3, and 4. The LSD results also indicated that reactor 2 had significantly higher performance in terms of TOC degradation, which statistically proved the observations made in Figure 4.13.

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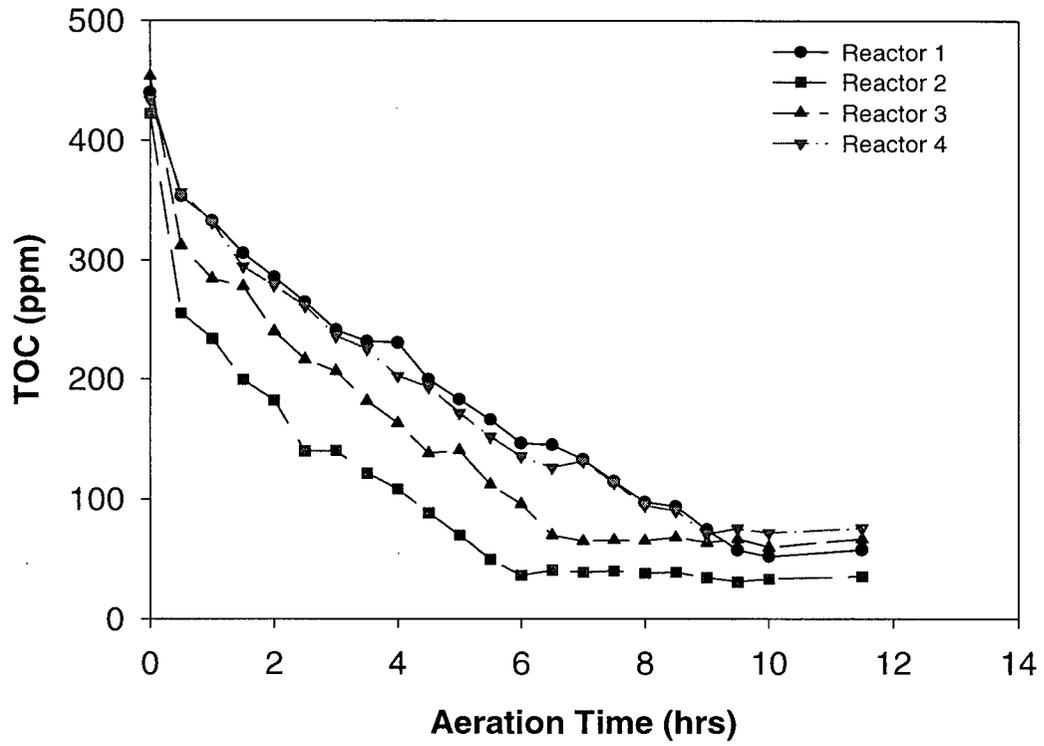
Figure 4.13 Continuous monitoring of TOC vs aeration time in four reactors.

Table 4.16.Summary of ANOVA for the four reactors

in track study of TOC degradation, DO and pH.

Sources of Variance	Reactors
df	3
C.V.	64.92
Root MSE	104.18
F value	2.99
p value	0.04

Dependent variable was concentration of TOC.
$\alpha = 0.05$

Table 4.17 Grouping of the four reactors in track study of TOC degradation, DO, and pH (Using fisher's Least Significant Difference methods)

REACTOR	Mean*
1	191.21 (A)
2	108.24 (B)
3	155.14 (AB)
4	187.35 (A)

*Means with the same letter are not significantly different.

4.5.2 The results of track study of pH, dissolved oxygen, and TOC in the suspended-growth aerobic SBRs

Environmental conditions pertaining to pH, temperature, dissolved oxygen concentration, and nutrient content have an important effect on the survival and growth of microorganisms. In this research, temperature was fixed at room temperature, which was about 20 °C. As depicted in Figure 4.14, the pH within the four reactors fluctuated between 6.7 to 7.8, which is the optimal pH range for most microbial growth. The degradation of organic compounds by microorganisms inside the reactors produced large amounts of CO_2, which, together with other small carbohydrate molecules (such as acetic acids) plays an important role in maintaining the pH of reactors. It was not necessary to add buffer to the aerobic SBRs even though raw brewery wastewater has a poor buffering capacity (Cronny, 1996).

The dissolved oxygen concentration, however, changed as aeration time increased, as did the concentration of TOC. Figure 4.14 shows that the dissolved oxygen concentration dropped slightly at the beginning of the reaction period after feeding with fresh wastewater. It decreased quickly from 9.7 mg/l to nearly zero (0.3 mg/l) within 6 hours. The start point of dissolved oxygen concentration in this figure was recorded when oxic feeding concluded. The reactors were saturated with DO by aeration at this point. The level of dissolved oxygen increased greatly at an aeration time of 6 –7 hours, and reached a platform at DO ~9.3 mg/l. Meanwhile, the concentration of TOC inside the reactors was reduced from 441.9 to 126.05 ppm in 11.5 hours, most of the organic carbon

compounds were spent within an aeration time 0-6 hours, and the remaining TOC continuously decreased as aeration time increased.

4.5.3 Relationship between TOC degradation and aeration time

An ANOVA and LSD procedure were run to statistically analyze the aeration time effects on TOC degradation. As shown in Table 4.18, aeration time had significant effect on TOC degradation. TOC degradation rate was calculated by Equation 4.3 for each of the groups (grouped based on the T-grouping):

$$\text{TOC degradation rate (ppm/min)} = \Delta[\text{TOC}]/ \Delta\text{Time} \qquad (4.3)$$

Where, $\Delta[\text{TOC}]$ is an increase of TOC with a certain ΔTime.

As shown in Table 4.19, the degradation rate of TOC (3.37 ppm/min) was much greater in the first half an hour after feeding with fresh wastewater. It varied between 0.40-0.82 ppm/min until aeration time reached 6.0 hours. The degradation rate of TOC was reduced to about 0.19-0.27 ppm/l as aeration proceeded past 6 hours.

Figure 4.15 shows the concentration of TOC vs aeration time at different influent concentrations of TOC in reactor 2. The trend for the two curves is alike, with the only difference being beginning point. At a higher influent concentrations (TOC = 1539.5 ppm), there was a sharp decrease of TOC after the first half an hour. This steep drop did not occur with lower influent TOC concentrations (TOC = 1109 ppm).

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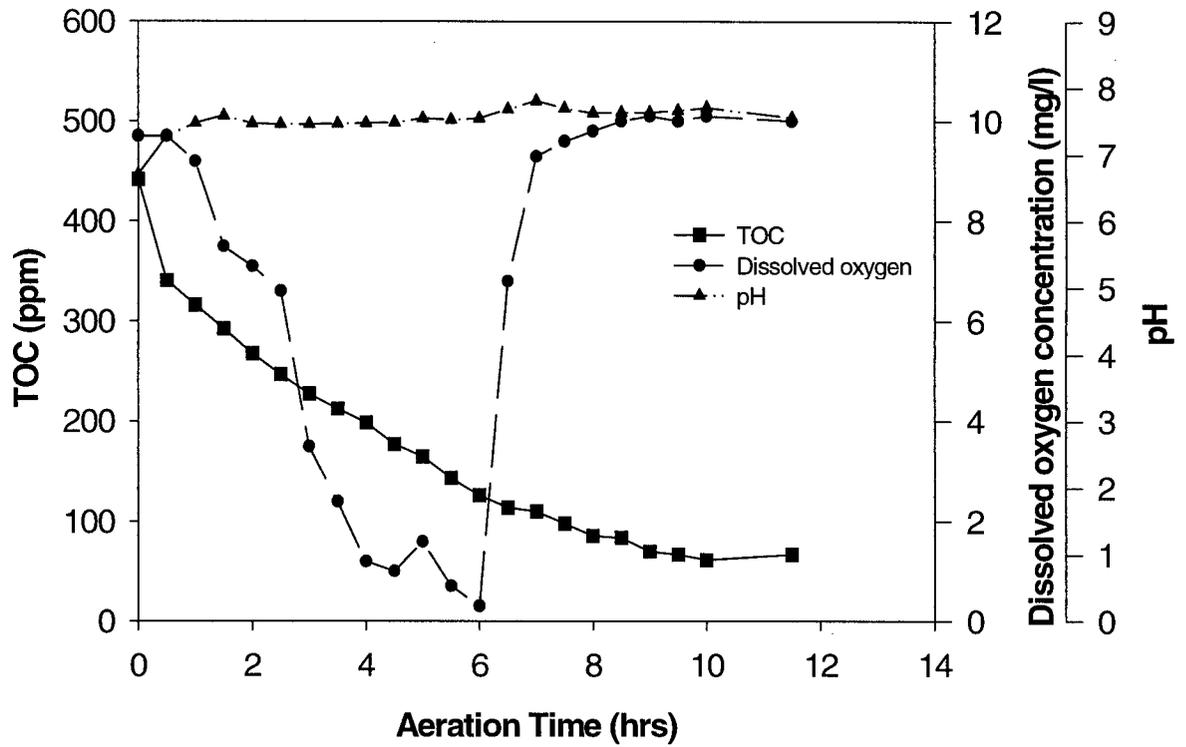
Figure 4.14 The plot of TOC (average of reactors #1, #3, and #4), dissolved oxygen, and pH vs aeration time in track study.
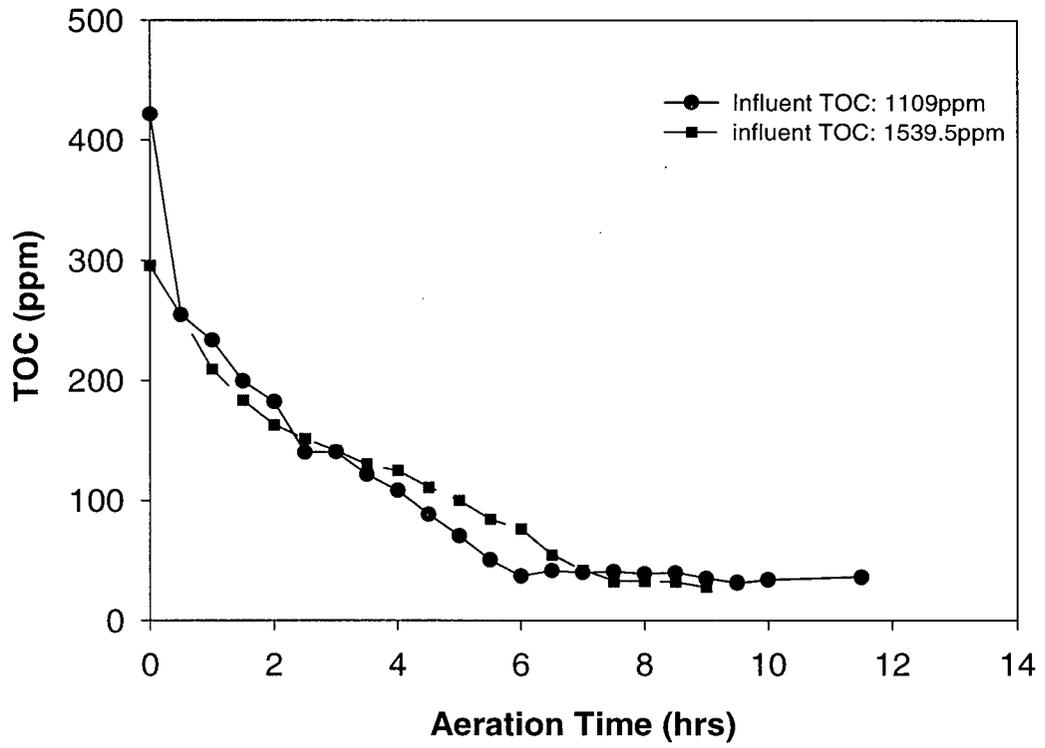
Figure 4.15 TOC vs aeration time under different influent concentrations in reactor 2.

Table 4.18. ANOVA analysis of aeration time effect in track study.

Sources of Variance	Aeration time
df	21
C.V.	26.28
Root MSE	42.19
F value	23.88
p value	0.0001

Dependent variable was concentration of TOC.
α=0.05

Table 4.19 The grouping of loading rate (based on the multi-comparison of the means).

T Grouping			Mean	TIME	TOC degradation rate (ppm/min)
A			441.90	0	3.37(A)
	B		340.70	0.5	0.82(B)
C	B		315.97	1	0.78(C)
C	D		292.57	1.5	0.82(D)
E	D		267.80	2	0.68(E)
E	F		247.30	2.5	0.65(F)
G	F		227.70	3	0.48(G)
G			212.57	3.5	
G	H		198.67	4	0.72(H)
I	H		177.10	4.5	0.40(I)
I	J		165.07	5	0.72(J)
K	J		143.43	5.5	0.49(K)
K	L		126.05	6	0.27(L)
K	L	M	113.97	6.5	0.25(M)
	L	M	110.10	7	
N	L	M	98.28	7.5	0.21(N)
N	O	M	85.74	8	0.16(O)
N	O	M	83.98	8.5	
N	O		69.81	9	
N	O		66.97	11.5	
N	O		66.72	9.5	
	O		61.40	10	

The stabilization of organic matter was achieved by the metabolic activity of microorganisms. As mentioned previously, nutrients and energy are used for microbial growth , and in the batch mode reactor, the changes in nutrient condition would therefore result in changes in microbial growth. A general bacterial growth pattern in pure culture is shown in Figure 4.16.

A lag phase occurred in the beginning. It represents the time required for the organism to acclimate to their new environment. During this lag growth phase, there is always an excessive amount of food surrounding the microorganisms, and the metabolic rate and growth is only a function of the ability of the microorganisms to process the substrate. This lag-growth phase was followed by a exponential growth phase, in which cell mass and cell number density increase exponentially with time, and nutrient concentrations are large. The next growth phase is stationary phase (also called declining growth phase), in which the cells have exhausted the substrate or nutrients necessary for growth. Thus the growth rate of the biomass declined because of limitations in the food supply, and the growth of new cells is offset by the death of old cells. In the death phase, the bacterial death rate exceeds the production of new cells.

Even though this growth model is the general bacterial growth pattern, it can be used to explain the growth of more complex microorganisms as well. The changes in TOC and dissolved oxygen concentrations may be explained by this growth model. As aeration time increased, the TOC concentration decreased because of the TOC degradation by microbial action. An increase of biomass and oxygen demanded by the oxidation activities of the vigorously growing microbes resulted in a decrease in the
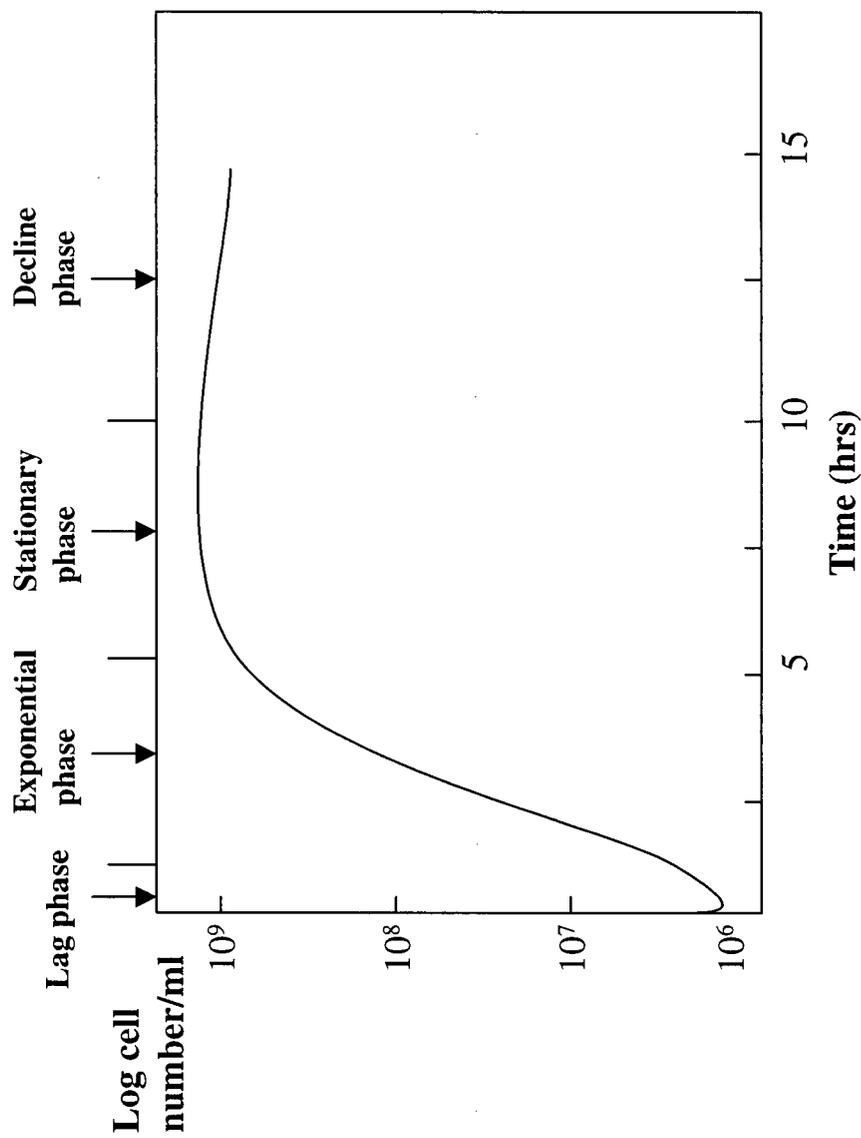
Figure 4.16 The typical bacterial growth pattern.

dissolved oxygen concentrations, since the air supply was fixed. At about 6 hours of aeration time, the oxygen consumption attained a maximum level, and the dissolved oxygen concentration reached its lowest point (0.3 mg/l), with the concentration of TOC about 126.05 ppm. The TOC degradation rate at this time was about 0.27 ppm/min (Table 4.19). This low degradation rate also indicated that the activity of microorganisms had slowed. The exhausted nutrients (in terms of TOC) and low dissolved oxygen concentration would impose an effect on the activities of microorganisms. After 6 hours' aeration, TOC and dissolved oxygen were consumed mainly for the purpose of the maintenance of cells. As continuous aeration proceeded, the DO increased dramatically due to a decrease in the oxygen requirement for the slower metabolic activities. By the end of aeration (at about 11.5 hours from the beginning), the residue of TOC detected was mostly composed of non-biodegradable organic compounds.

4.5.4 Sludge production

Throughout this study, it was noticed that the increase of sludge was quite slow. SRT was set at 28 days for most of the time. Unfortunately, biomass analysis was not carried out parallel to the continuous monitoring of TOC and dissolved oxygen. However, MLSS data from 0 hours of aeration (the beginning) and 11.5 hours of aeration (the ending) were collected and were used to calculated the sludge production rate.

Sludge production rate was calculated by the following equation:

$$\text{Sludge production rate} = \Delta \text{MLSS} / \Delta \text{TOC} \qquad (4.4)$$

Where, ΔMLSS was the net increase of mixed liquor suspended solids, and ΔTOC represented the TOC removed. As listed in Table 4.20, the average of sludge production rate was 0.16. This sludge yield was much lower than for other activated sludge treatment systems. A similar result of low sludge production in an SBR has been reported. In Dubeski's (1993) study, it was proven that aerobic SBR for CTMP wastewater treatment produced as much as one quarter the sludge of a typical activated sludge system, both on a COD and BOD_5 basis. This discovery indicated an important virtue of an aerobic SBR compared to other activated sludge systems.

Table 4.20 Sludge production in the suspended-growth SBR.

	MLSS (mg/l)	TOC (ppm)
initial	4078.93	391.83
End of aeration	4130.31±236.46	64.49±50.70
Sludge production rate	0.16	

As we know, SBR is conducted in a semi-batch mode. Since the nutrient is expected to be exhausted before next cycle, there may be a period of nutrient limitation or even depletion inside of the reactor. Under these conditions, the microorganisms would undergo a endogenous metabolism, and they are forced to metabolize their own protoplasm without replacement as the concentration of available food is at a minimum. This results in expansion of the biomass is expended, and results in relatively low sludge yields.

Conclusions and Recommendations

5.1 Conclusions

This study demonstrated that it was feasible to use an aerobic sequencing batch reactor system to treat brewery wastewater without any need for supplemental nitrogen-nutrients or phosphorous-nutrients. The performance of the suspended-growth SBR was significantly higher than that of the attached-growth SBR. In the suspended-growth SBR, over 94% removal of TOC, BOD5, COD was attained. The effects of HRT and loading rate on the treatment efficiencies were significant under both growth conditions. Greater sensitivity to changes in HRT and loading rate were seen in the attached-growth SBR. In terms of the microbial population, it was observed that the floc-forming bacteria were dominant in suspended-growth reactors, while in attached-growth reactors, filamentous bacteria flourished.

To further study the effects of HRT and loading rate on the performance of the suspended-growth SBR, factorial experiments and response surface analysis were carried out. An optimum (maximum) TOC removal (as dependent response variable) was predicted based on this response surface, with HRT and loading rate at 1.44 days and 3122 ppm/day, respectively. For the optimum (maximum) suspended solids removal, the predicted critical value of HRT and loading rate were 1.32 days and 13.84 g/l/day, respectively. It was noted when comparing the two eigenvectors of response surfaces (TOC removal and suspended solids removal), that TOC removal was more affected by

changes in HRT, while the loading rate was more important than HRT for suspended solids removal.

It was found that the dissolved oxygen concentration changed as the aeration time increased, even though the aeration rate was fixed in the track study. It was inferred from this that TOC degradation and the changes in dissolved oxygen concentration in the suspended-growth SBR were related to the microbial metabolic activities. The pH was maintained at the optimal range for microbial growth, which was between 6.7 to 7.8.

It was also noticed that the sludge production rate was low in the aerobic SBR systems, which was about 0.16.

5.2 Recommendations

In this study, the Ringlace was used to set up the attached-growth SBRs. The strands of the Ringlace were 5 cm apart. The diameter of biomass flocs attached on the Ringlace was 1.5 to 3.0 cm. Results indicated that the Ringlace with its attached sludge blocked adequate aeration, and the efficiency of the oxygen transfer was thereby decreased. It is therefore suggested that the distance of the Ringlaces should be larger than 5 cm.

It was found that the concentration of MLSS was one of the most important factors affecting the performance of SBR systems. It is therefore suggested that a further study should be done on the behavior of MLSS. Since the microbial population plays a dominant role in biological treatment systems, the amplification of certain species would greatly enhance treatment efficiency.

Another important consideration which should be taken into account is the sludge production rate. Given that the sludge production rate was low (0.16) in this study, this likely is one of the advantages of an aerobic SBR system over other systems used in the treatment of brewery wastewater. This should also be explored in further studies.

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APPENIX

Contents

Tables   <u>Pages</u>

85

Table A-1. TS and VS removal in suspended- and attached-growth SBRs
under a series HRT

| | | | Growth Type | | | |
| | | | Suspended-growth | | Attached-growth | |
HRT	Solids	Stat.	Effluent(mg/l)	Removal	Effluent(mg/l)	Removal
0.56 TS		N	11	11	12	12
		MIN	555.00	0.54	757.00	0.29
		MAX	788.00	0.70	1065.00	0.58
		MEAN	650.64	0.61	887.75	0.47
		STD	82.88	0.06	97.81	0.10
	VS	N	12	12	12	12
		MIN	328.00	0.04	370.00	0.00
		MAX	520.00	0.34	523.00	0.25
		MEAN	404.58	0.18	435.00	0.10
		STD	64.04	0.10	47.36	0.14
0.81 TS		N	8	8	8	8
		MIN	545.00	0.54	735.00	0.47
		MAX	712.00	0.71	965.00	0.60
		MEAN	624.13	0.63	802.50	0.52
		STD	62.47	0.06	70.39	0.05
	VS	N	8	8	8	8
		MIN	328.00	0.00	388.00	0.00
		MAX	462.00	0.28	493.00	0.23
		MEAN	395.88	0.13	425.75	0.08
		STD	37.77	0.12	33.94	0.10
1.06 TS		N	8	8	8	8
		MIN	465.00	0.58	550.00	0.47
		MAX	580.00	0.70	748.00	0.68
		MEAN	545.00	0.65	647.50	0.58
		STD	41.69	0.05	77.98	0.07
	VS	N	8	8	8	8
		MIN	133.00	0.00	277.00	0.00
		MAX	347.00	0.53	375.00	0.32
		MEAN	273.00	0.24	312.00	0.13
		STD	89.47	0.17	34.94	0.14
1.56 TS		N	4	4	4	4
		MIN	587.00	0.63	670.00	0.58
		MAX	612.00	0.64	693.00	0.59
		MEAN	597.00	0.63	681.50	0.58
		STD	12.25	0.01	11.68	0.01

(Table A-1)

			Growth type			
			Suspended-growth		Attached-growth	
HRT	Solids	Stat.	Effluent(mg/l)	Removal	Effluent(mg/l)	Removal
	VS	N	4	4	4	4
		MIN	395.00	0.00	387.00	0.00
		MAX	457.00	0.06	438.00	0.08
		MEAN	427.25	0.03	413.75	0.03
		STD	33.33	0.03	21.27	0.04
3.06	TS	N	12	12	12	12
		MIN	433.00	0.66	450.00	0.62
		MAX	745.00	0.80	830.00	0.79
		MEAN	575.25	0.74	658.00	0.70
		STD	96.91	0.04	105.18	0.05
	VS	N	12	12	12	12
		MIN	212.00	0.56	210.00	0.60
		MAX	365.00	0.75	333.00	0.75
		MEAN	297.00	0.65	295.67	0.65
		STD	42.24	0.05	33.11	0.04
6.06	TS	N	10	10	10	10
		MIN	502.00	0.66	485.00	0.65
		MAX	750.00	0.77	772.00	0.78
		MEAN	627.60	0.71	647.60	0.70
		STD	90.21	0.04	81.34	0.04
	VS	N	10	10	10	10
		MIN	253.00	0.55	160.00	0.58
		MAX	377.00	0.70	352.00	0.81
		MEAN	323.70	0.61	308.60	0.63
		STD	40.94	0.05	55.15	0.07

Table A2. TS and VS removal in suspended- and attached-growth SBRs
under a series loading rate

| SOLIDS | Loading rate (mg/l/day) | Stat. | Growth type | | | |
| | | | Suspended-growth | | Attached-growth | |
			Effluent (mg/l)	Removal	Effluent (mg/l)	Removal
TS	4321	N	10	10	10	10
		MIN	502.00	0.66	485.00	0.65
		MAX	750.00	0.77	772.00	0.78
		MEAN	627.60	0.71	647.60	0.70
		STD	90.21	0.04	81.34	0.04
	8555	N	12	12	12	12
		MIN	433.00	0.66	450.00	0.62
		MAX	745.00	0.80	830.00	0.79
		MEAN	575.25	0.74	658.00	0.70
		STD	96.91	0.04	105.18	0.05
	12538	N	4	4	4	4
		MIN	587.00	0.63	670.00	0.58
		MAX	612.00	0.64	693.00	0.59
		MEAN	597.00	0.63	681.50	0.58
		STD	12.25	0.01	11.68	0.01
	15627	N	4	4	4	4
		MIN	465.00	0.58	550.00	0.47
		MAX	580.00	0.66	735.00	0.60
		MEAN	533.75	0.61	640.50	0.54
		STD	56.48	0.04	90.72	0.07
	19687	N	4	4	4	4
		MIN	525.00	0.67	563.00	0.57
		MAX	575.00	0.70	748.00	0.68
		MEAN	556.25	0.68	654.50	0.62
		STD	23.00	0.01	76.33	0.04
	22746	N	4	4	4	4
		MIN	547.00	0.54	763.00	0.47
		MAX	712.00	0.64	810.00	0.51
		MEAN	638.50	0.59	785.75	0.49
		STD	69.64	0.05	19.21	0.01
	27306	N	4	4	4	4
		MIN	545.00	0.63	735.00	0.48
		MAX	692.00	0.71	965.00	0.60
		MEAN	609.75	0.67	819.25	0.56
		STD	60.86	0.03	102.20	0.06

89

(Table A-2)

			Growth type			
			Suspended-growth		Attached-growth	
SOLIDS	Loading rate (mg/l/day)	Stat.	Effluent (mg/l)	Removal	Effluent (mg/l)	Removal
	32128	N	4	4	4	4
		MIN	575.00	0.56	890.00	0.29
		MAX	665.00	0.62	1065.00	0.41
		MEAN	612.25	0.59	977.00	0.35
		STD	37.87	0.02	71.64	0.05
	36181	N	4	4	4	4
		MIN	680.00	0.54	757.00	0.43
		MAX	788.00	0.60	975.00	0.55
		MEAN	743.25	0.56	858.25	0.49
		STD	50.62	0.03	102.98	0.06
	39540	N	4	4	4	4
		MIN	555.00	0.68	780.00	0.51
		MAX	590.00	0.70	903.00	0.58
		MEAN	578.33	0.69	828.00	0.55
		STD	20.21	0.01	52.93	0.03
VS	1658	N	10	10	10	10
		MIN	253.00	0.55	160.00	0.58
		MAX	377.00	0.70	352.00	0.81
		MEAN	323.70	0.61	308.60	0.63
		STD	40.94	0.05	55.15	0.07
	3152	N	4	4	4	4
		MIN	133.00	0.00	277.00	0.00
		MAX	290.00	0.53	375.00	0.01
		MEAN	202.75	0.28	304.75	0.00
		STD	74.17	0.25	46.95	0.00
	3226	N	4	4	4	4
		MIN	395.00	0.00	387.00	0.00
		MAX	457.00	0.06	438.00	0.08
		MEAN	427.25	0.03	413.75	0.03
		STD	33.33	0.03	21.27	0.04
	3283	N	12	12	12	12
		MIN	212.00	0.56	210.00	0.60
		MAX	365.00	0.75	333.00	0.75
		MEAN	297.00	0.65	295.67	0.65
		STD	42.24	0.05	33.11	0.04

(Table A-2)

| SOLIDS Loading rate (mg/l/day) | Stat. | Growth type | | | |
| | | Suspended-growth | | Attached-growth | |
		Effluent (mg/l)	Removal	Effluent (mg/l)	Removal
4855	N	4	4	4	4
	MIN	338.00	0.19	292.00	0.19
	MAX	347.00	0.22	347.00	0.32
	MEAN	343.25	0.20	319.25	0.26
	STD	3.86	0.01	22.47	0.05
5428	N	4	4	4	4
	MIN	328.00	0.00	388.00	0.00
	MAX	418.00	0.11	427.00	0.00
	MEAN	383.75	0.03	405.75	0.00
	STD	40.10	0.05	20.69	0.00
7847	N	4	4	4	4
	MIN	385.00	0.13	410.00	0.07
	MAX	462.00	0.28	493.00	0.23
	MEAN	408.00	0.23	445.75	0.16
	STD	36.45	0.07	34.53	0.06
10585	N	4	4	4	4
	MIN	328.00	0.24	370.00	0.10
	MAX	377.00	0.34	448.00	0.25
	MEAN	356.00	0.28	396.75	0.20
	STD	20.51	0.04	34.90	0.07
11492.00	N	4	4	4	4
	MIN	427.00	0.04	410.00	0.03
	MAX	520.00	0.21	523.00	0.24
	MEAN	479.75	0.11	451.00	0.16
	STD	39.68	0.07	62.55	0.12

Table A-3. The effects of the HRT and the loading rate on TS removal
in suspended-growth SBRs

HRT (day)	Loading rate (mg/l/day)	Stat.	TS(Effluent)	TS removal
0.56	15493	N	4	4
		MIN	463.00	0.27
		MAX	533.00	0.36
		MEAN	508.25	0.30
		STD	31.70	0.04
	23040	N	4	4
		MIN	560.00	0.39
		MAX	660.00	0.48
		MEAN	595.50	0.45
		STD	46.02	0.04
	26133	N	4	4
		MIN	515.00	0.48
		MAX	640.00	0.58
		MEAN	582.50	0.52
		STD	52.36	0.04
	31360	N	4	4
		MIN	475.00	0.57
		MAX	587.00	0.59
		MEAN	535.50	0.58
		STD	52.13	0.01
1.06	16885	N	8	8
		MIN	525.00	0.60
		MAX	643.00	0.68
		MEAN	591.63	0.63
		STD	47.25	0.03
	21106	N	8	8
		MIN	553.00	0.62
		MAX	713.00	0.70
		MEAN	661.63	0.65
		STD	50.74	0.03
2.06	4225	N	4	4
		MIN	460.00	0.21
		MAX	577.00	0.37
		MEAN	498.75	0.31
		STD	53.44	0.07

(Table A-3)

HRT (day)	Loading rate (mg/l/day)	Stat.	TS(Effluent)	TS removal
	6284	N	4	4
		MIN	547.00	0.32
		MAX	735.00	0.49
		MEAN	654.25	0.39
		STD	87.34	0.08
	7127	N	4	4
		MIN	458.00	0.47
		MAX	650.00	0.63
		MEAN	570.00	0.53
		STD	80.87	0.07
	8553	N	4	4
		MIN	532.00	0.51
		MAX	718.00	0.64
		MEAN	633.00	0.57
		STD	77.34	0.05

Table A-4. Response surface analysis of TS removal in suspended-growth SBRs

(a) Parameters of TS removal response surface	
Variable	TS percentage removal
Response Mean	0.518458
Root MS	0.059270
R^2	0.8121
Coef. of Variation	11.4319

(b) Critical value		
Factor	Coded	Uncoded
HRT	3.011363	3.568522
LOADING	2.144313	46885
Predicted value at stationary point *		1.541512

(c) Eigenvectors		
Eigenvalues	HRT	LOADING
-0.055647	0.929299	0.369328
-0.146275	-0.369328	0.929299

* Stationary point is a maximum.

Table A-5. The effects of the HRT and the loading rate on VS removal
in suspended-growth SBRs

HRT (day)	Loading rate (mg/l/day)	Stat.	VS (Effluent)	VS removal
0.56	3147	N	4	4
		MIN	192.00	0.00
		MAX	295.00	0.00
		MEAN	258.50	0.00
		STD	45.57	0.00
	4987	N	4	4
		MIN	250.00	0.00
		MAX	325.00	0.00
		MEAN	280.50	0.00
		STD	35.09	0.00
	6293	N	4	4
		MIN	190.00	0.00
		MAX	333.00	0.36
		MEAN	275.25	0.13
		STD	70.36	0.17
	7307	N	4	4
		MIN	202.00	0.13
		MAX	297.00	0.41
		MEAN	252.25	0.26
		STD	47.33	0.14
1.06	3078	N	8	8
		MIN	287.00	0.00
		MAX	383.00	0.00
		MEAN	338.13	0.00
		STD	31.81	0.00
	4504	N	8	8
		MIN	350.00	0.00
		MAX	427.00	0.12
		MEAN	386.75	0.04
		STD	26.00	0.05
2.06	858	N	4	4
		MIN	265.00	0.00
		MAX	313.00	0.00
		MEAN	277.50	0.00
		STD	23.69	0.00

(Table A-5)

HRT (day)	Loading rate (mg/l/day)	Stat.	VS (Effluent)	VS removal
	1360	N	4	4
		MIN	272.00	0.00
		MAX	357.00	0.00
		MEAN	320.50	0.00
		STD	36.61	0.00
	1716	N	4	4
		MIN	192.00	0.00
		MAX	362.00	0.35
		MEAN	280.25	0.12
		STD	72.10	0.16
	1993	N	4	4
		MIN	230.00	0.08
		MAX	315.00	0.33
		MEAN	281.75	0.18
		STD	37.40	0.11

Table A-6. The effects of HRT and the loading rate on VSS removal
           in suspended-growth SBRs

HRT (day)	Loading rate (mg/l/day)	Stat.	VSS (Effluent)	VSS removal
0.56	5	N	8	8
		MIN	0.00	0.00
		MAX	4.00	1.00
		MEAN	1.00	0.50
		STD	1.41	0.53
	48	N	8	8
		MIN	0.00	0.00
		MAX	4.00	1.00
		MEAN	1.06	0.63
		STD	1.37	0.40
	192	N	8	8
		MIN	1.00	0.00
		MAX	10.00	0.89
		MEAN	4.50	0.51
		STD	3.17	0.33
	315	N	8	8
		MIN	1.00	0.76
		MAX	3.50	0.93
		MEAN	2.25	0.85
		STD	0.89	0.06
	1344	N	8	8
		MIN	3.50	0.75
		MAX	16.00	0.94
		MEAN	8.63	0.86
		STD	5.10	0.08
	1349	N	8	8
		MIN	3.50	0.87
		MAX	8.50	0.95
		MEAN	5.81	0.91
		STD	1.75	0.03
	1771	N	8	8
		MIN	12.00	0.76
		MAX	20.00	0.86
		MEAN	15.50	0.81
		STD	2.62	0.03

(Table A-6)

HRT (day)	Loading rate (mg/l/day)	Stat.	VSS (Effluent)	VSS removal
	1829	N	8	8
		MIN	4.00	0.90
		MAX	8.50	0.95
		MEAN	5.88	0.93
		STD	1.60	0.02
1.06	836	N	16	16
		MIN	0.00	0.60
		MAX	29.50	1.00
		MEAN	7.47	0.90
		STD	7.98	0.11
	1132	N	16	16
		MIN	0.00	0.88
		MAX	12.00	1.00
		MEAN	4.69	0.95
		STD	4.00	0.04
	1304	N	16	16
		MIN	0.00	0.91
		MAX	10.00	1.00
		MEAN	4.44	0.96
		STD	3.45	0.03
	1313	N	16	16
		MIN	0.00	0.44
		MAX	65.00	1.00
		MEAN	13.75	0.88
		STD	16.03	0.14
2.06	1	N	8	8
		MIN	1.00	0.00
		MAX	7.00	0.00
		MEAN	5.13	0.00
		STD	1.89	0.00
	13	N	8	8
		MIN	0.00	0.33
		MAX	1.50	1.00
		MEAN	0.44	0.81
		STD	0.50	0.22
	52	N	8	8
		MIN	0.00	0.50
		MAX	4.50	1.00
		MEAN	1.56	0.83
		STD	1.45	0.16

(Table A-6)

HRT (day)	Loading rate (mg/l/day)	Stat.	VSS (Effluent)	VSS removal
	86	N	8	8
		MIN	2.00	0.19
		MAX	12.00	0.86
		MEAN	6.38	0.57
		STD	3.58	0.24
	367	N	8	8
		MIN	0.00	0.93
		MAX	4.50	1.00
		MEAN	1.76	0.97
		STD	1.82	0.03
	368	N	8	8
		MIN	1.70	0.94
		MAX	4.00	0.97
		MEAN	2.99	0.95
		STD	0.80	0.01
	483	N	8	8
		MIN	2.00	0.93
		MAX	6.00	0.98
		MEAN	3.83	0.95
		STD	1.32	0.02
	499	N	8	8
		MIN	1.00	0.95
		MAX	4.50	0.99
		MEAN	2.73	0.97
		STD	1.26	0.01

Table A-7. The effects of the HRT and loaidng rate on the BOD_5 removal
in suspended-growth SBRs

HRT (day)	Loading rate* (mg/l/day)	Stat.	BOD_5 (effluent)** (mg/l)	BOD_5 removal
0.56	25063	N	8	8
		MIN	8.67	0.98
		MAX	19.98	0.99
		MEAN	14.58	0.99
		STD	4.20	0.00
	34147	N	8	8
		MIN	17.48	0.96
		MAX	63.48	0.99
		MEAN	45.27	0.97
		STD	18.35	0.01
	35283	N	8	8
		MIN	7.96	0.98
		MAX	35.04	1.00
		MEAN	23.09	0.99
		STD	10.41	0.01
	47042	N	8	8
		MIN	38.58	0.97
		MAX	69.01	0.98
		MEAN	57.07	0.97
		STD	11.88	0.01
1.06	13549	N	8	8
		MIN	2.89	0.99
		MAX	11.56	1.00
		MEAN	6.86	0.99
		STD	3.04	0.00
	14990	N	8	8
		MIN	3.62	0.99
		MAX	14.91	1.00
		MEAN	7.77	0.99
		STD	3.71	0.00
2.06	9313	N	8	8
		MIN	9.25	0.97
		MAX	42.40	0.99
		MEAN	20.30	0.99
		STD	13.84	0.01

(Table A-7)

HRT (day)	Loading rate* (mg/l/day)	_STAT_	BOD$_5$ (effluent)** (mg/l)	BOD$_5$ removal
	12829	N	8	8
		MIN	7.79	0.96
		MAX	78.92	1.00
		MEAN	37.87	0.98
		STD	27.50	0.01

*   The loading rate of BOD$_5$ was calculated by the ratio TOC/ BOD$_5$ = 0.78;

** The concentration of BOD$_5$ in the effluent was calculated by the ratio TOC/ BOD$_5$ = 4.86.

Table A-8. Response surface analysis of COD removal in suspended-growth SBRs

(a) Parameters of COD removal response surface	
Variable	COD percentage removal
Response Mean	0.921036
Root MSE	0.036663
R^2	0.5247
Coef. of Variation	3.9806

(b) Critical Value		
Factor	Coded	Uncoded
HRT	0.170224	1.437668
Loading rate	-1.086927	7628.141586
Predicted value at stationary point *		0.981236

(c) Eigenvectors		
Eigenvalues	HRT	LOADING
-0.009846	-0.044490	0.999010
-0.071003	0.999010	0.044490

* Stationary point is a maximum.

Table A-9. The effects of the HRT and loading rate on the COD removal
in suspended-growth SBRs

HRT (day)	Loading rate* (mg/l/day)	Stat.	COD (effluent)** (mg/l)	COD removal
0.56	39541	N	8	8
		MIN	193.80	0.82
		MAX	338.30	0.90
		MEAN	274.34	0.85
		STD	52.73	0.03
	44430	N	8	8
		MIN	87.80	0.90
		MAX	202.30	0.96
		MEAN	147.66	0.93
		STD	42.49	0.02
	53720	N	8	8
		MIN	184.70	0.81
		MAX	480.10	0.93
		MEAN	344.80	0.86
		STD	103.83	0.04
	60534	N	8	8
		MIN	176.90	0.77
		MAX	642.70	0.94
		MEAN	458.29	0.84
		STD	185.74	0.07
	62548	N	8	8
		MIN	80.60	0.88
		MAX	354.80	0.97
		MEAN	233.81	0.92
		STD	105.36	0.04
	64011	N	8	8
		MIN	429.00	0.82
		MAX	528.00	0.86
		MEAN	485.74	0.84
		STD	37.64	0.01
	74643	N	8	8
		MIN	340.10	0.85
		MAX	532.60	0.90
		MEAN	440.75	0.87
		STD	60.24	0.02

(Table A-9)

HRT (day)	Loading rate* (mg/l/day)	Stat.	COD (effluent)** (mg/l)	COD removal
	83393	N	8	8
		MIN	390.60	0.82
		MAX	698.80	0.90
		MEAN	577.83	0.85
		STD	120.28	0.03
1.06	22211	N	8	8
		MIN	36.00	0.92
		MAX	162.20	0.98
		MEAN	116.00	0.94
		STD	52.50	0.03
	24018	N	8	8
		MIN	29.30	0.95
		MAX	117.00	0.99
		MEAN	69.49	0.97
		STD	30.80	0.01
	24045	N	8	8
		MIN	37.10	0.97
		MAX	70.30	0.98
		MEAN	51.73	0.98
		STD	14.55	0.01
	25880	N	8	8
		MIN	50.40	0.97
		MAX	77.70	0.98
		MEAN	67.20	0.97
		STD	11.34	0.00
	26573	N	8.00	8.00
		MIN	36.70	0.94
		MAX	150.90	0.98
		MEAN	78.69	0.97
		STD	37.53	0.02
	28441	N	8	8
		MIN	80.80	0.94
		MAX	143.10	0.97
		MEAN	110.23	0.96
		STD	22.60	0.01
	32173	N	8	8
		MIN	60.00	0.96
		MAX	127.90	0.98
		MEAN	94.30	0.97
		STD	26.39	0.01

(Table A-9)

HRT (day)	Loading rate* (mg/l/day)	Stat.	COD (effluent)** (mg/l)	COD removal
	39516	N	8	8
		MIN	115.50	0.92
		MAX	266.30	0.97
		MEAN	185.29	0.95
		STD	62.81	0.02
2.06	10784	N	8	8
		MIN	74.80	0.90
		MAX	185.30	0.96
		MEAN	140.68	0.92
		STD	40.31	0.02
	12118	N	8	8
		MIN	118.30	0.90
		MAX	215.60	0.94
		MEAN	161.95	0.92
		STD	36.64	0.02
	14652	N	8	8
		MIN	69.30	0.92
		MAX	203.10	0.97
		MEAN	139.38	0.94
		STD	49.59	0.02
	16509	N	8	8
		MIN	93.70	0.85
		MAX	429.30	0.97
		MEAN	205.55	0.93
		STD	140.11	0.05
	17059	N	8	8
		MIN	139.80	0.90
		MAX	292.90	0.95
		MEAN	227.79	0.92
		STD	65.08	0.02
	17457	N	8	8
		MIN	57.80	0.89
		MAX	324.50	0.98
		MEAN	179.46	0.94
		STD	108.44	0.04

(Table A-9)

HRT (day)	Loading rate * (mg/l/day)	Stat.	COD (effluent)** (mg/l)	COD removal
	20357	N	8	8
		MIN	70.60	0.92
		MAX	272.70	0.98
		MEAN	132.74	0.96
		STD	83.79	0.02
	22743	N	8	8
		MIN	78.80	0.80
		MAX	799.10	0.98
		MEAN	383.43	0.90
		STD	278.47	0.07

* The loading rate of COD was calculated by the ratio TOC/COD = 0.44;
**The concentration of COD in the effluent was calculated by the ratio TOC/COD=0.48.

Table A-10 Analytical methods and instrumentation

| Parameter	Methods	Instrument/Reference
TKN	Digested with H2SO4 and K2SO4	Technicon AutoanalyzerII
TP	Digested with H2SO4 and K2SO4	Technicon Autoanalyzer II
NH3-N	Automatic phenate methods	Technicon Autoanalyzer II
NO3-N/NO2-N	Calorimetric automated cadmium reduction methods	Technicon Autoanalyzer II
PO4-P	Automated ascorbic acid reduction methods	Technicon Autoanalyzer II
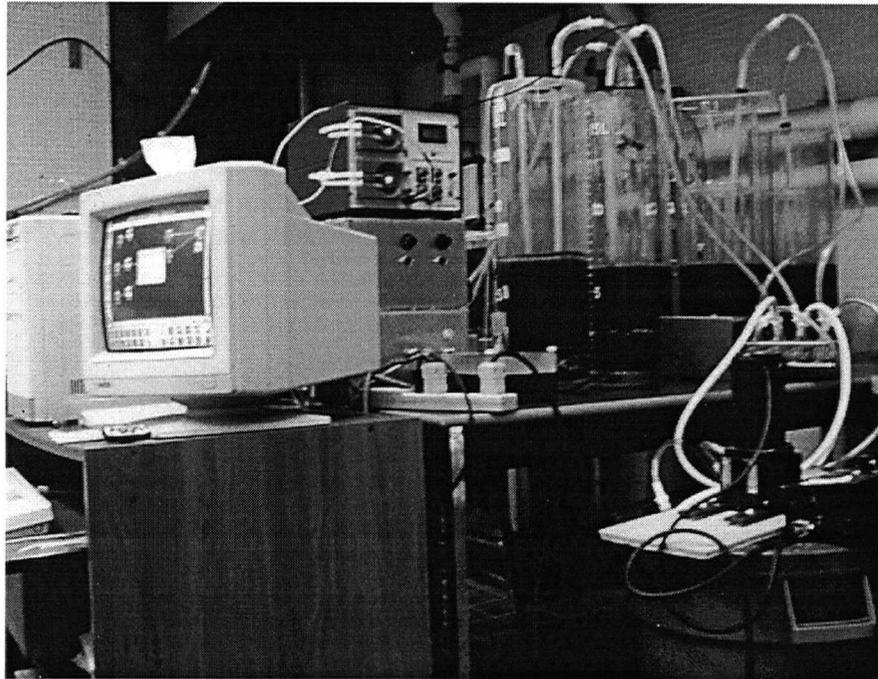
Figure A-1. The picture of the experimental set-up.
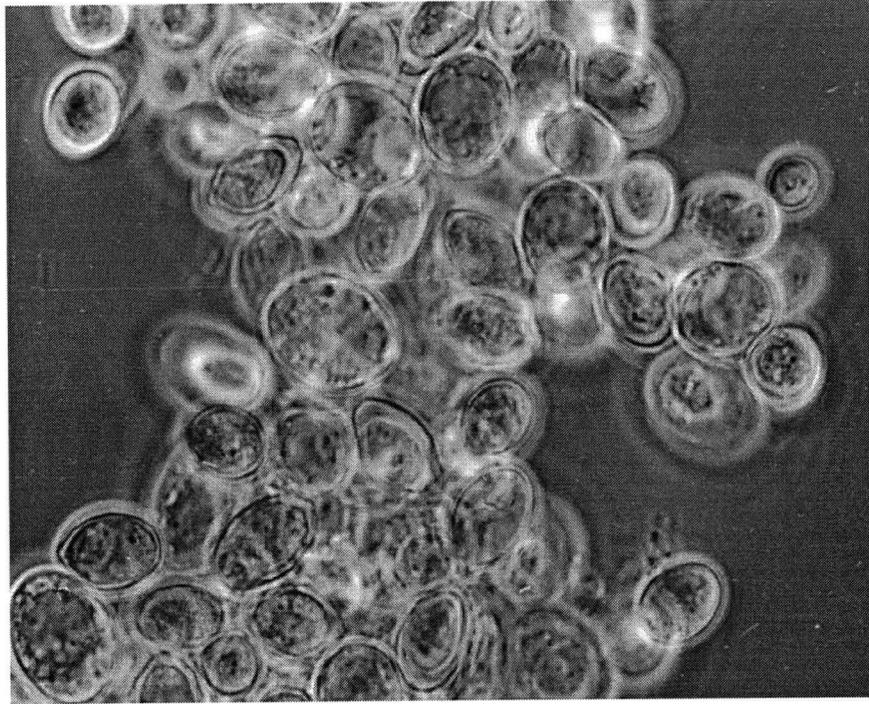
Figure A-2. The picture of organisms (yeast spores) in

the raw brewery wastewater (40X).