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Steady-state operation of bench-scale activated sludge process using phenol and 2-chlorophenol as substrates

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ABSTRACT

Title of Thesis: Steady-State Operation of Bench-Scale Activated Sludge Process Using Phenol and 2-Chlorophenol as Substrates

Jeffrey Caputi, Master of Science in Environmental Engineering (Toxicology Option), 1987

Thesis directed by: Dr. Gordon A. Lewandowski
Professor of Chemical Engineering

Experiments were conducted to determine whether a continuous process similar to the common activated sludge process could be operated at a relative steady state with phenol and 2-chlorophenol as the sole carbon sources. After a number of unsuccessful trials, process conditions were developed which allowed for operation at a relative steady state with respect to the concentration of microorganisms in the reactor and the concentration of phenol and 2-chlorophenol in the effluent.

Phenol and 2-chlorophenol were removed at better than 99 percent efficiency on a continuous basis. The mixed liquor grown under the conditions of the experiment was highly filamentous and exhibited poor settling characteristics. However, reasonably good clarification was achieved by providing a long enough residence time in the clarifier.

STEADY-STATE OPERATION OF BENCH-SCALE ACTIVATED SLUDGE PROCESS USING PHENOL AND 2-CHLOROPHENOL AS SUBSTRATES

by
Jeffrey Caputi

Thesis submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
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APPROVAL SHEET

Title of Thesis: Steady-State Operation of Bench-Scale Activated Sludge Process Using Phenol and 2-Chlorophenol as Substrates

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Phenol is present in oil refinery, polymeric resin production, and coking plant effluents [6,22]. It is toxic to fish at low concentrations (5-25 mg/l) and imparts an objectionable taste to water at much lower concentrations [22]. Furthermore, chlorination reactions that take place during the disinfection of drinking water sources or wastewater effluents containing phenol can result in the formation of highly toxic chlorophenols [11]. Phenol and 2-chlorophenol both are experimental carcinogens [42].

Biological treatment processes are now being used to treat contaminated groundwater at hazardous waste sites [46, 48] as well as industrial effluents containing phenolic compounds [31,38]. These processes are preferred to treatment processes that simply transfer toxic compounds from water to some other medium (air stripping, carbon adsorption) because biological treatment transforms the compounds into the simpler substances of which they are composed. Most often, this results in detoxification of the compound [3].

Previous experiments in our laboratory have shown phenol and 2-chlorophenol can be effectively removed from dilute aqueous systems in batch reactors employing mixed populations of microorganisms [29]. The need to develop operational criteria for continuous flow systems exists because most real-life processes are continuous.

Experiments were conducted to determine whether a continuous process similar to the common activated sludge process could be operated at a relative steady state with respect to solids concentration in the reactor and substrate removal efficiency. The results of five representative runs are presented herein.

II. LITERATURE REVIEW

The activated sludge process and its modifications has been described in a number of texts [9,13,32,50]. The conventional activated sludge process is commonly employed for secondary treatment of municipal wastewater because it provides the best balance among capital investment, operating costs, and removal efficiency [50].

Busch has described the essential elements of a bench-scale activated sludge process [10]. These include: 1) continuous flow for uniform organic loading, 2) metered aeration, 3) positive suspended solids control, and 4) a hydraulic loading similar to full scale requirements.

Alexander has reported on biodegradation of synthetic organic chemicals [2,3,4]. He studied a number of compounds which were resistant to biodegradation (recalcitrant) and listed 15 reasons why a compound may be resistant:

- Nonexistence of an active organism
- Violation of comparative biochemistry
- Violation of enzyme specificity
- Lack of sufficient energy or carbon for growth
- Lack of an essential nutrient
- Exceeding of microbial tolerance to environmental factors
- Toxicity of substrate or products of its metabolism

- Inhibition or inactivation of extracellular enzyme
- Failure of chemical to penetrate the cell
- Concentration of substrate in aqueous solution is too low
- Lack of induction or requisite enzymes
- Need for different organisms
- Inaccessibility of substrate
- Complexing of substrate with resistant organic or polyaromatic compounds
- Inaccessibility of site on substrate acted on enzymatically

Many authors have reported on the biodegradability of phenol [6,20,22,23,27,28,31,34,36,37,38,39,47,51]. A number of them have reported steady state operation of bench-scale systems using phenol as the primary or sole carbon source [6,7,20,23,35,37,39,40]. Removal efficiencies of greater than 99 percent have been reported for a variety of influent phenol concentrations and reactor designs [23,28,31,36,38,43, 47,51].

The biodegradability of 2-chlorophenol has been studied as well [5,8,12,18,21,26,28,30,36,43,48,51]. 2-Chlorophenol is generally considered less toxic and more easily degraded than the more highly chlorinated phenols [5,22,26,30,36].

Some authors have reported that 2-chlorophenol (ortho-chlorophenol) is also less toxic than the meta and para

isomers [26,30,36]. Experiments have shown that 2-chlorophenol is completely degraded at low concentrations, on the order of 10 mg/l, but is only partially degraded at higher concentrations, on the order of 100 mg/l [18,52].

Beltrame, et al. [6] operated a continuous reactor with recycle at steady state using phenol as the sole carbon source. The activated sludge was taken from a municipal/industrial wastewater treatment plant near Milan, Italy and adapted to phenol for four months in batch reactors.

A C:N:P ratio of 100:10:2 was used with influent phenol concentrations (So) of 180 and 360 mg/l. Trace nutrients were added to the feed as well.

Multiple runs were performed at hydraulic detention times (H) of 1.56 and 6.25 hours. Effluent phenol concentrations (S) ranged from 105 to 301 mg/l when So was 360 mg/l, H was 1.56 hours, and the mixed liquor suspended solids concentration (X) ranged from 238 to 3237 mg/l.

When H was increased to 6.25 hours, S ranged from 113 to 238 mg/l and X ranged from 280 to 746 mg/l. When So was decreased to 180 mg/l and H remained at 6.25 hours, S ranged from 33 to 105 mg/l and X ranged from 47 to 263 mg/l.

The authors reported the phenol removal rate was higher for the lower value of So. They also reported no substrate inhibition at these concentrations and concluded that processes employing sludge recycle can treat higher phenol concentrations than can be treated in batch reactors or

continuous reactors without recycle. It was noted that the mixed liquor grown on phenol exhibited poor settling characteristics, but the suspended solids measured in the effluent were always less than one percent of the mixed liquor suspended solids.

Pawlowsky et al. [34,35] conducted experiments in both batch and continuous reactors using phenol as the sole carbon source. Activated sludge was obtained from the Batavia, New York wastewater treatment plant and mixed with soil. Nitrogen, phosphorus, and trace nutrients were supplied in the feed.

The mixed liquor was continuously fed 100 mg/l phenol in a chemostat. Temperature was maintained at 28 C, and pH was maintained at 6.6. When H was set at 6 hours, the predominant form of microorganisms was spherical and rod shaped bacteria. When H was set at 4 hours, filamentous bacteria predominated.

These populations were then used in subsequent batch studies where phenol was introduced at concentrations ranging from 100 to 800 mg/l. The authors reported that phenol was inhibitory at the higher concentrations but that the filamentous forms are less affected by the inhibitory effect of phenol.

Holladay et al. [23] studied the biodegradation of phenolic waste liquors in stirred-tank, packed-bed, and fluidized-bed bioreactors. The initial microbial population for the activated sludge process (stirred-tank bioreactor)

was obtained from Bethlehem Steel Corporation's activated sludge tank, where it had been used to treat coal-coking effluents for several years.

The stirred-tank reactor was equipped with an external clarifier and a sludge recycle pump. The aeration tank volume was 2.5 liters. A one-liter Imhoff cone was utilized as a clarifier. Temperature was controlled at 32 C. Air was supplied at a rate of 3 l/min.

The reactor was operated for about four months with a variety of waste streams used as the feed. Nitrogen, phosphorus, and trace nutrients were added to the influent. Phenol conversion in the stirred-tank reactor was consistently greater than 99 percent for undisrupted steady-state periods of operation. These conversions were obtained for phenol concentrations ranging from 100 to 1400 mg/l and retention times from 11.7 to 79.5 hours.

The authors found that a feed concentration of 1400 mg/l phenol could be routinely processed to effluent levels of 1 mg/l or less. However, it was also found that phenol degradation rates were higher in the packed-bed and fluidized-bed reactors and that the stirred-tank reactor was most affected by shock loadings.

Rozich et al. [39] studied the biodegradation of phenol in a continuous reactor with recycle, but employed procedures to maintain a constant suspended solids concentration in the recycle sludge. Steady state data was obtained for seven

runs, although the authors noted that the nature of the substrate caused operational problems not routinely encountered with more easily biodegradable carbon sources.

In all seven of the runs, only trace amounts of phenol were observed in the effluent. The clarifier underflow sludge was described as "light and fluffy but not filamentous." High concentrations of suspended solids were present in the effluent.

Based on the results obtained from the runs at various detention times, the authors concluded that failure of a system treating an inhibitory substrate can be expected to occur more abruptly as the detention time is decreased than for a non-inhibitory substrate. They recommended that H be maintained above 8 hours for all but very low influent concentrations of phenol unless provisions are made to thicken the recycled sludge.

In summary, microbial degradation of phenol and chlorophenols has been observed in many laboratory studies in which these compounds represented the primary carbon sources. Continuous flow processes have been operated using phenol as the primary carbon source. However, the sludge grown on phenol exhibited poor settling characteristics and the systems were vulnerable to shock loadings. There is some disagreement regarding the inhibitory effects of phenol and the effectiveness of biological treatment in removing phenolic compounds from dilute aqueous systems.

III. EXPERIMENTAL APPARATUS AND PROCEDURES

A. INFLUENT FEED PREPARATION

Table 1 lists the elemental composition for the bacterium Escherichia coli, a widely-occurring and well studied species. The elemental composition of microorganisms varies somewhat, depending on the environmental conditions and species of microorganism [19].

The ratio of carbon:nitrogen:phosphorus shown in Table 1 was used in preparing the influent feed. However, the proportion of carbon was doubled to account for the fact that about 50 percent of the available carbon is used to provide energy to the organism (catabolism), while the other 50 percent is used for synthesis (anabolism) [19]. Therefore, the influent feed had a C:N:P ratio of 100:14:3.

Influent feed was prepared in 50 liter batches. The carbon source consisted of either phenol or a combination of phenol and 2-chlorophenol. Nitrogen and phosphorus were provided by adding appropriate amounts of ammonium carbonate and ammonium phosphate to the influent feed. The amount of each compound was determined according to the weight of carbon, nitrogen, and phosphorus per gram-weight of the compound:

Phenol	-	76.57% C
2-Chlorophenol	-	56.06% C
Ammonium carbonate	-	29.16% N
Ammonium phosphate	-	12.18% N, 26.93% P

All chemicals were Reagent Grade and met A.C.S. specifications. The desired concentration of phenol and 2-chlorophenol was set, and the other compounds were added in amounts corresponding to the C:N:P ratio. The chemicals were mixed in tap water so trace nutrients would be provided.

B. REACTOR SETUP AND OPERATION

The reactor setup initially used was the Bio-Oxidation System, sold by Horizon Ecology Company of Chicago, Illinois. The main components of the system are a console and a reactor. The Bio-Oxidation Console, Model 5551-20, comes equipped with the following:

- Variable-speed gear motor
- Fixed-speed gear motor
- Air pump
- Rotameter

Two Masterflex Model 7017 peristaltic pumps and one Masterflex Model 7018 peristaltic pump are provided for influent flow, effluent flow, and solids removal.

Figure 1 shows the reactor components. The reactor is a six-liter glass cone set in a metal stand. A two-liter glass cone sits inside the outer cone, and a glass clarifier tube is suspended in the inner cone, which is open at both ends. Three air difussers are situated near the bottom of the outer

cone.

Figure 2 depicts the reactor setup, showing direction of air and liquid flow. Air is introduced to the system near the bottom of the outer cone. Air bubbles are released at the liquid surface as the liquid then flows down the inner cone to recirculate. The effluent rises under quiescent conditions in the cylindrical clarifier tube in the center of the inner cone, while mixed liquor suspended solids settle into the recirculating flow. The recirculated flow rate is large compared to the flow through the system, providing a completely mixed system in terms of liquid residence time [24].

Run 1

The first run was attempted using the original setup as described above. The operating parameters for the system were as follows:

Q = Influent flow rate = 16 ml/min

Qe = Effluent flow rate = 16 ml/min

V = Volume of mixed liquor = 6 liters

So = Influent substrate concentration

= 500 mg/l phenol

H = Hydraulic detention time = V/Q = 6.25 hr

L = Volumetric loading = So*Q/V = 80 mg/l*hr

A = Air flow rate = 10 SCFH (4.7 l/min)

Run 2

The second run was attempted without modifications to the original setup. The operating parameters were as follows:

Q = 16 ml/min

Qe = 16 ml/min

V = 6 liters

So = 250 mg/l phenol

H = 6.25 hr

L = 40 mg/l*hr

A = 5 SCFH (2.4 l/min)

The influent phenol concentration was reduced from 500 mg/l to 250 mg/l. The influent flow rate was not changed, so the volumetric loading was also reduced by 50 percent. The air flow rate was reduced from 10 to 5 SCFH in an effort to prevent turbulent conditions from occurring in the settling zone of the system and reduce the concentration of solids in the clarifier overflow.

Run 3

For the third run, the reactor setup was modified to better approximate a typical activated sludge process. The inner cone and clarifier tube were removed from the system. An external clarifier was constructed as follows:

1. A one-liter oil funnel was suspended above a large (20 liter) open-top container.
2. The aeration tank overflow was routed to the top of the clarifier via a peristaltic pump driven by the fixed-speed motor.
3. A sludge recycle line was set up by drawing off the bottom of the clarifier via a peristaltic pump driven by the variable speed motor. This allowed for changing the rate of sludge recycle.
4. A separate variable-speed peristaltic pump (Sage Instruments Model 375A, Division of Orion Research, Inc., Cambridge, Massachusetts) was added to the system to deliver the influent feed.

The modifications were designed to improve settling and allow for better solids control. Solids could be removed from the system through the drain tube at the bottom of the aeration tank.

The operating parameters for Run 3 were as follows:

Q = 25 ml/min

Qe = 25 ml/min

Qr = Recycle flow rate = 15 ml/min

Qr/Q = Recycle ratio = 0.6

V = 6 liters

Vc = Clarifier volume = 1 liter

So = 250 mg/l phenol

H = 4.0 hr

L = 62.5 mg/l*hr

A = 10 SCFH

Qw = Solids removal rate = 250 ml/day

Run 4

For the fourth run, the oil funnel which served as the clarifier in Run 3 was replaced by an Imhoff cone. The Imhoff cone has a steeper slope, and it was felt that this would improve settling in the clarifier. The result of improved settling would be twofold:

1. The solids concentration in the recycle sludge would increase.
2. The solids concentration in the clarifier overflow would decrease.

The operating parameters for Run 4 were as follows:

Q = 25 ml/min

Qe = 25 mg/l

Qr = 30 ml/min

Qr/Q = 1.2

V = 6 liters

Vc = 1 liter

So = 250 mg/l phenol

H = 4.0 hr

L = 62.5 mg/l*hr

A = 10 SCFH

Qw = 250 ml/day

The recycle ratio was increased from 0.6 to 1.2 in an effort to reduce the height of the sludge blanket in the clarifier. The other parameters were the same as in Run 3.

Run 5

The clarifier volume was increased from one liter to 2.8 liters for the fifth run. This was done by attaching a 1.8-liter plastic cylinder to the top of the Imhoff cone.

The operating parameters are summarized below:

Q = 8 ml/min

Qe = 8 ml/min

Qr = 16 ml/min

Qr/Q = 2

V = 4.2 liters

Vc = 2.8 liters

So = 250 mg/l phenol (Day 1-21), 225 mg/l phenol plus 25 mg/l 2-chlorophenol (Day 22-31)

H = 8.75 hr

L = 28.6 mg/l*hr

A = 10 SCFH

Qw = 500 ml/day

The hydraulic detention time was increased and the volumetric loading decreased by reducing both the influent flow rate and the volume of mixed liquor in the aeration tank. The influent substrate concentration remained at 250 mg/l; however, the phenol concentration was reduced to 225 mg/l and 25 mg/l 2-chlorophenol was added to the influent feed after 21 days. The recycle ratio was increased further from 1.2 to 2. The solids wasting rate was increased to 500 ml/day.

C. HYDRAULIC AND MIXING CHARACTERISTICS

Mixing characteristics are most important to aerobic biological basins. Two hydraulic phenomena which can limit the degree of mixing are sections of dead space or plug flow [1].

Approximately 63 percent of the dye added to a completely mixed system can be recovered after one theoretical detention time. The theoretical detention time (T) is equal to the volume of fluid in the reactor divided by the rate of flow through the system. The percent recovery is determined as follows:

$$\text{Percent Recovery} = 100\ [1-\exp(-t/T)]$$

where t is the actual measured time interval and T is the theoretical detention time.

The amount of dead space in a completely-mixed reactor is determined by observing at what fraction of a detention time 63 percent of the dye is recovered. The fraction of dead space is equal to the remaining fraction. For example, if 63 percent of the dye is recovered at t/T = 0.75, then the fraction of dead space would be equal to 0.25 (25 percent).

In the plug flow case, the curve always passes through 63 percent dye recovery at t/T = 1.0, but the curve can originate at various points on the abscissa. For example, if the fraction of plug flow were equal to 10 percent, the dye recovery curve would originate at t/T = 0.10.

The following procedure was used to determine the fractions of dead space and plug flow in the system:

1. Known concentrations of Evans Blue dye were prepared in distilled water. Standards were prepared with dye concentrations equal to 0.1, 0.5, 1, 5, and 10 mg/l.
2. The system was cleansed and rinsed with distilled water.
3. The reactor was filled with a known volume of distilled water, approximately 6 liters.
4. The air flow rate was set at 5 SCFH (2.4 l/min) with the rotameter.

5. The liquid flow rate was measured and set constant at 25 ml/min by adjusting the variable-speed motor.
6. One ml of 1000 mg/l dye solution was injected into the influent feed line for each liter of reactor volume.
7. The effluent was sampled initially and at predetermined intervals for approximately two detention times.
8. Standards and samples were analyzed on a visible range spectrophotometer (Gilford Model 1367X5, Oberlin, Ohio) with the wavelength set at 600 nm.
9. A standard curve was prepared by plotting the known dye concentration versus the measured absorbance readings.
10. A dye recovery curve was prepared by plotting the ratio of measured dye concentrations to the initial dye concentration versus the ratio of measured time values to the theoretical detention time.
11. The t/T value for 63 percent dye recovery was determined, and the fractions of dead space and plug flow were estimated.

D. OXYGEN TRANSFER COEFFICIENT

Most aerobic biological systems are operated under turbulent flow conditions. Under these conditions, transfer of oxygen from the gas phase to the liquid phase is a

function of the overall transfer coefficient (KLA) and the oxygen deficit [1,15]. The oxygen transfer coefficient was determined in accordance with Method 208 as described in Standard Methods for the Examination of Water and Wastewater [45].

The procedure employed was as follows:

1. A dissolved oxygen probe (Model 97-08, Orion Research, Cambridge, Massachusetts) was calibrated in accordance with the manufacturer's instructions.
2. The reactor was filled to a volume of six liters with tap water. The dissolved oxygen (DO) and temperature in the reactor were measured.
3. The dissolved oxygen saturation concentration (Cs) was read from tables [41].
4. Water in the reactor was deoxygenated by adding 10 mg/l sodium sulfite per 1 mg/l dissolved oxygen and 1 mg/l cobalt chloride catalyst. The air supply was turned on just long enough to mix the chemicals and the DO was measured with the probe.
5. The air supply was set at 5 SCFH (2.4 l/min) with the rotameter, and the DO in the clarifier tube was measured at timed intervals until a constant value was obtained.
6. The value of KLA was determined by plotting the natural logarithm of (Cs-C) versus time where C is

the DO at time (t) beginning with the first nonzero value for C. The slope of the line is equal to KLA.

7. The value for KLA was adjusted to 20 C according to the equation:

$$(KLA) @ T = (KLA) @ 20 * 1.024(\exp(T-20)).$$

8. The experiment was repeated replacing tap water with mixed liquor obtained from the Passaic Valley Sewerage Commissioners wastewater treatment plant in Newark, New Jersey.
9. The ratio (alpha) of KLA for mixed liquor to that for tap water was determined, as well as the ratio (beta) of DO saturation concentrations.

E. TEMPERATURE AND pH

All experiments were conducted at room temperature. Temperature was determined by submersing a mercury thermometer into the reactor. The thermometer was calibrated to one degree Celsius, and readings were rounded to the nearest degree. The thermometer was continuously immersed in the aeration tank.

The pH of the activated sludge was monitored with a combination pH electrode (Orion) and a digital ionanalyzer (Orion Model 501 or 701A). The pH was not adjusted or maintained by the addition of buffers beyond those present in

the influent feed.

Buffer solutions of pH 4, 7, and 10 were used for calibrating the ionanalyzer immediately before measurements. The electrode was immersed in the aeration tank, and readings were not taken until the output on the digital meter was constant. The meter provides readings to 0.01 standard pH units. Measurements were rounded to the nearest 0.1 pH units.

F. DISSOLVED OXYGEN AND OXYGEN CONSUMPTION RATE

The dissolved oxygen concentration in the aeration tank was measured by immersing a dissolved oxygen probe (Orion Model 97-08) into the tank. The probe was connected to a digital ionanalyzer (Orion Model 501 or 701A) and was calibrated in accordance with the manufacturer's instructions. Readings were not recorded until the output on the ionanalyzer was constant.

Oxygen consumption rate (OCR) was determined in accordance with Method 213-A in Standard Methods for the Examination of Water and Wastewater [45]. The procedure was as follows:

1. Enough sample to fill a 300-ml BOD bottle was removed from the aeration tank, placed in a one-liter bottle, and saturated with oxygen by shaking vigorously.
2. A 300-ml BOD bottle was filled with the mixed liquor

sample.

3. A dissolved oxygen probe (Orion Model 97-08) was placed in the BOD bottle along with a magnetic stirring bar, and the bottle was set on a magnetic stirrer.
4. The initial DO reading was recorded after the meter reading stabilized, and a stopwatch was started.
5. Measurements were taken for a period of 15 minutes or until DO readings no longer changed with time.
6. The observed DO readings were plotted versus time, and the slope of the line of best fit was taken as the OCR.

G. SUSPENDED SOLIDS

Suspended solids are frequently used as an index of microorganism concentration [9,19,32]. The common procedure for determining suspended solids is described in Standard Methods for the Examination of Water and Wastewater, Method 209-D [45]. Because of difficulty in filtering the mixed liquor cultured during the experiments, an alternate procedure was developed. The procedure employed was as follows:

1. Aluminum weighing dishes were stored in a dessicator and weighed to one onethousandth of a gram on an electronic analytical balance (Denver Instrument

Company Model DTL350, Denver, Colorado) before use.

2. Ten milliliters of sample were placed in the aluminum dishes with a pipette. Samples were collected in triplicate.
3. The aluminum dishes containing the sample were placed in an oven in which the temperature was maintained at 103 to 105 C. Samples remained in the oven for at least 2-4 hours.
4. The dried samples were allowed to cool to room temperature in a dessicator. The samples were then reweighed on the analytical balance.
5. The suspended solids concentration was determined by subtracting the initial (tare) weight from the final weight. The three values for each sample were averaged, and the result was rounded to the nearest 100 mg/l. Values which were greater or less than the middle value by more than 25 percent of the middle value were considered outliers and were not used in calculating the average.

Mixed liquor suspended solids (MLSS) samples were collected directly from the aeration tank. For Runs 1 and 2, samples were collected from the outer cone because this area of the reactor represented the aeration tank in the typical activated sludge process.

Samples of suspended solids in the effluent were taken from the container in which the clarifier overflow was collected. The container was emptied on a daily basis, and its contents were stirred before collecting samples to ensure that solids were evenly distributed and a representative sample was obtained.

Samples from the sludge recycle line were obtained by temporarily disconnecting the sludge return from the aeration tank and collecting the flow in a beaker. The contents of the beaker were then stirred, and the sample was transferred from the beaker to the weighing dish with a 10 ml pipette.

H. EFFLUENT SUBSTRATE CONCENTRATION

Effluent substrate concentration was determined by chromatographic analysis. Samples were collected in 20-ml glass vials containing 0.5 ml of 20,000 mg/l copper sulfate. The copper sulfate is an effective biocide, and previous experiments conducted in our laboratory have shown that this concentration is sufficient to prevent any further biodegradation of substrate in the sample [17]. Samples were stored at 8 C in the dark prior to analysis.

Runs 1-4

Samples were collected from the aeration tank. Solids were concentrated by centrifuging for 5 minutes at 1200 rpm, and the supernatant was transferred to the vials with a 10-ml

volumetric pipette. In addition to the 0.5 ml of copper sulfate, the vials contained 0.5 ml of 1000 mg/l thymol, which was used as an internal standard in the analyses. The total volume of each sample vial was 11 ml, and the thymol concentration was 45.45 mg/l.

Samples were analyzed on a gas chromatograph (Tracor Model 565). Samples were introduced to the column with an autosampler (Tracor Model 770). The following information pertains to the analyses:

Injection volume - 2 microliters
Injections per sample - 3
Analysis time - 3 minutes
Injection port temperature - 300 C
Column type - 10% SP-2100 on 100/120 Supelcoport, 6 feet by 1/8 inch stainless steel (Supelco, Bellefonte, Pennsylvania)
Column temperature - 160 C (isothermal)
Detector type - flame ionization
Detector temperature - 300 C
Recorder type - integrator (Hewlett Packard Model 3390A)

Standard concentrations of phenol (25 mg/l) and thymol (45.45 mg/l) were used to calibrate the integrator. Samples were then transferred to 1 ml vials, placed in the auto-

sampler, and the analyzed. Distilled water was injected between samples to prevent carryover from the previous sample.

The concentration of phenol in each sample was determined directly by the integrator. Three values were obtained for each sample. A single value was determined by taking the arithmetic mean of the three values. Values which were greater or less than the middle value by more than 25 percent of the middle value were not used in calculating the average.

Run 5

Samples were collected from the top of the clarifier. The samples were placed directly into 20-ml vials containing 1 ml of copper sulfate. The samples were not centrifuged. Thymol was not added to the samples. The samples were stored at 8 C in the dark prior to analysis.

A sample of the influent feed was collected on Day 9. The sample was collected after about 90 percent of the feed had been used, and the remaining solution in the influent container was three days old. The sample was stored in the same manner as the other Run 5 samples.

The analytical equipment and operating conditions were the same as for Runs 1-4, except the autosampler was not employed. Standards and samples were introduced to the GC by manual injection with a 10-microliter syringe. The injected

volume was 2 microliters.

An internal standard was not employed. Standards containing known concentrations of phenol and 2-chlorophenol were prepared, and five point standard curves were developed by plotting the response (peak area) versus the concentration of each substrate.

The substrate was changed from 250 mg/l phenol to 225 mg/l phenol and 25 mg/l 2-chlorophenol after 21 days. Previous experiments in our laboratory have shown that phenol and 2-chlorophenol are difficult to separate by gas chromatography and that their peaks will overlap at high concentrations [17]. Because effluent substrate concentrations were not expected to be high in Run 5, it was felt that separation would be adequate using this procedure. Standards were analyzed individually under the same conditions to avoid separation problems.

IV. RESULTS AND DISCUSSION

A. HYDRAULIC AND MIXING CHARACTERISTICS

The standard curve obtained for Evans Blue dye is shown in Figure 3. The correlation coefficient for the line of best fit was equal to 0.99998, indicating the response was linear over the range of concentrations analyzed.

Experimental results are listed in Table 2. The dye recovery curve is shown in Figure 4. As the curve indicates, 63 percent recovery occurred at t/T equal to 0.98. Therefore, the fraction of dead space in the reactor was equal to approximately 0.02 (2 percent). The curve originates at t/T = 0, indicating that sections of plug flow do not effect mixing in the system.

Since this experiment was performed with water and not mixed liquor, it cannot be assumed that complete mixing occurs for the activated sludge process. However, the results demonstrate that the reactor is well designed with respect to hydraulic and mixing characteristics.

B. OXYGEN TRANSFER COEFFICIENT

Experimental data is presented in Tables 3 and 4. Curves used in determining KLA are shown in Figures 5 and 6. For tap water, KLA was equal to 0.365/min at 21 C. When adjusted to 20 C, the value of KLA is 0.356/min. For the mixed liquor, KLA was equal to 0.100/min, which is equal to 0.091/min when

adjusted to 20 C.

The results show a significantly lower transfer rate for the mixed liquor than for the tap water. Since there where no changes in the aeration system, the difference is most likely due to the characteristics of the mixed liquor.

One possibility for the difference could be the presence of high chloride concentrations in the mixed liquor. Oxygen dissolves less well in water which is bonded to ions [44]. The mixed liquor used in the experiment was obtained during the month of April, when chloride concentrations may be high from road salting.

Another possible explanation for the lower transfer rate for the mixed liquor is that oxygen was being consumed during the experiment. The mixed liquor sample was collected from the aeration tank of the treatment plant, before secondary settling. Oxygen is consumed as the higher organisms feed on bacteria in the mixed culture, commonly referred to as endogenous decay. Hwang et al. [25] found that KLA varied inversely with microbial OCR in experiments performed on mixed liquor.

The results for alpha and beta are as follows:

$$\text{alpha} = 0.091 / 0.356 = 0.255$$

$$\text{beta} = 7.80 / 9.04 = 0.863$$

These values can be used in sizing aeration equipment when designing large-scale processes from bench-scale pilot tests. However, because they depend upon the installed aeration equipment, the best method to evaluate aerators is to conduct full-scale field tests [13].

C. TEMPERATURE AND pH

Temperature and pH data collected during the five runs are listed in Tables 5 - 9. Temperature rarely changed by more than one degree Celsius from one day to the next. The largest difference over the course of an entire run was 6 C in Run 2. The effect of temperature on the rate of reaction for microorganisms is well studied and has been found to increase with increasing temperature, doubling with about every 10 C rise in temperature until some limiting temperature is reached [32].

Temperature changes were small and gradual enough to avoid exerting a noticable effect on the mixed liquor suspended solids and effluent substrate concentrations. Tighter control of temperature is important in conducting kinetic experiments, where degradation rates are to be com- pared for different conditions in the system.

The results of pH measurements taken during the five runs are presented in Tables 5 - 9. The pH was always between 6.5 and 7.9. In Runs 1, 2, and 5, the pH of the mixed liquor was 7.6 or higher when received from the treatment plant but

dropped during the initial stages of the experiment before stabilizing in the 6.8 to 7.2 range. The initial pH was lower in Runs 3 and 4 (6.6 and 6.9, respectively) and in both cases it remained in that range throughout the experiment.

Some authors have reported the need for pH adjustment in experiments of this type [35,37]. Generally, the optimum pH for bacteria is between 6.5 and 7.5, while lower pH levels are more favorable to fungi [19,32] and may promote filamentous growth [16].

D. DISSOLVED OXYGEN AND OXYGEN CONSUMPTION RATE

The dissolved oxygen concentration did not vary significantly over the course of the experiments. Results of the measurements are presented in Tables 10-14. In order to ensure that DO is not a limiting substrate, the literature [13] indicates that its minimum concentration must be between 0.5 to 2.0 mg/l, depending on the characteristics of the wastewater. The lowest DO concentration measured in the present study was 4.7 mg/l in Run 3.

Oxygen consumption rate data are listed in Tables 10-14 and graphed in Figures 7-11. Figure 7 shows a sharp increase in OCR between 6 and 12 hours, indicating an increase in microbial activity. Figures 8-11 all show a sharp increase from Day 0 (initial measurement) to Day 1 (measurement after approximately 24 hours).

The sudden increase in respiration is expected because the mixed liquor was not acclimated to the substrate in any of the runs. The low oxygen consumption rates during the early stages of the experiments exemplify the lag phase of the standard growth curve [19,32].

In Runs 1-4, the OCR remained at a high rate (0.8 to 1.4 mg/l/min) after rising from the initial rate, except for the final readings in Runs 1 and 3. In these cases the OCR was zero, indicting the microorganisms were no longer active.

In Run 5, the OCR increased to 0.98 mg/l/min after one day but then decreased over the next four days before leveling off at 0.32 mg/l/min. The OCR ranged from a low of 0.26 to a high of 0.70 mg/l/min over the remainder of the experiment. The wide range of rates may be due to the non-homogeneity of the mixed liquor, which was observed to change dramatically during the experiment.

Most of the readings were in the 0.26 to 0.40 mg/l/min range. The addition of 2-chlorophenol to the influent feed on Day 22 did not exert a noticable effect on the oxygen consumption rate.

E. SUSPENDED SOLIDS

Run 1

Mixed liquor suspended solids results for Run 1 are listed in Table 15 and plotted in Figure 12. The results indicate a sharp decrease in mixed liquor suspended solids. Solids were observed in the effluent (clarifier overflow) but were not measured. Settling appeared to be hindered by turbulent conditions in the inner cone. The only solids lost from the system were those in the effluent.

Run 2

The mixed liquor suspended solids concentration did not fall as sharply in Run 2 as in Run 1 as evidenced by the results shown in Table 16 and Figure 13. After conducting tests in which the air flow rate was varied and the dissolved oxygen in the system was measured, it was determined that the air flow rate could be reduced from 10 to 5 SCFH without significantly lowering the DO level in the reactor.

The effect of the reduced airflow was to improve settling in the inner cone, but not to the degree necessary to prevent solids washout. Rather than attempt to further reduce the air flow, and allow the DO to fall to levels which may be unfavorable to the desired microorganisms, the system was modified to provide a clarifier separate from the aeration tank.

Run 3

Table 17 lists suspended solids data for Run 3. The solids concentration in the aeration tank remained stable for the first four days. On Day 5, the solids level in the clarifier overflow rose sharply. This was accompanied by a decline in the solids concentration in both the aeration tank and the sludge recycle. These trends are illustrated in Figures 14-16.

Solids in the clarifier were resting on (and possibly adhering to) the walls and not settling into the bottom section where they could be concentrated and recycled. Eventually, the solids in the upper section of the clarifier began to overflow into the effluent. It was decided that the clarifier shape was at fault, and the oil funnel which was used for the clarifier in this run was replaced by an Imhoff Cone.

Run 4

Suspended solids data for Run 4 are listed in Table 18 and graphed in Figures 17-19. The results are similar to those obtained in Run 3, except the rise in effluent suspended solids occurred on Day 6, and the associated decrease in mixed liquor and recycle sludge suspended solids was not observed until Day 7.

It was now apparent that the clarifier was being overloaded. The hydraulic detention time (Hc) for the clarifier

was calculated according to the equation:

$$Hc = Vc / Q$$

where Vc is the clarifier volume and Q is the flow rate to the clarifier. For Run 4, Hc was equal to 0.67 hr. Typical values for secondary clarifiers following conventional activated sludge processes are 2-6 hours [32]. In addition, the recycle ratio was below the recommended value of 1.5 for small-scale plants [32].

Run 5

The results of suspended solids determinations for Run 5 are presented in Table 19. The clarifier detention time was increased to 5.8 hr by changes in both the flow rate and clarifier volume.

The process was operated for 31 days at a relative steady state with respect to suspended solids. The suspended solids concentrations in the aeration tank are plotted in Figure 20. The MLSS ranged from 1800-2800 mg/l but never changed by more than 400 mg/l from one day to the next. Daily fluctuations usually were in the 0-200 mg/l range.

Figure 21 shows the recycle sludge suspended solids concentrations over the 31 day period. The solids concentration dropped over the first 10-12 days before leveling off at 2800-3200 mg/l. By Day 10, the biomass in the system

had changed color from the characteristic brown to a light-yellow or straw color. The texture was almost gelatinous. The material did not form flocs and did not settle well in the clarifier.

A sample of the mixed liquor was collected on Day 17, and the microbial characteristics were examined using standard plating techniques. The predominant microbial and fungal genera are listed in Table 20. The MLSS was determined to be highly filamentous with non-branching filaments 1.0 to 1.5 micrometers in width.

Bulking is associated with excessive growth of filamentous microorganisms and may have contributed to the settling problems encountered in the earlier runs [16,32,49]. Strom [49] has noted that good clarification can be achieved under highly filamentous conditions as long as the sludge blanket does not escape over the clarifier weirs.

While excessive filaments hinder the close approach of floc during settling, and can decrease the apparent solids density, clarification can still be achieved if the clarifier is large enough due to the filtering action of the extended filamentous networks [49]. The effluent suspended solids data, which is graphed in Figure 22, demonstrate that good clarification was achieved throughout the course of Run 5. Values reported as <100 mg/l in Table 19 are shown as zero on the graph.

The causes of filamentous growth are not well understood. However, the following conditions may contribute to filamentous growth [16,32,49]:

- wide fluctuations in pH
- low dissolved oxygen concentrations
- deficiency in one or more inorganic nutrients
- low organic loading (food-to-microorganism ratio)

The pH did not fluctuate during the experiment, and the dissolved oxygen concentration was always above 5 mg/l. It is not expected that nitrogen or phosphorus was deficient. However, it is possible that there was a deficiency of some trace nutrient such as sulfur or iron.

The food-to-microorganism ratio is calculated according to the equation:

$$F/M = So / H * X$$

where So is the influent substrate concentration, H is the hydraulic detention time, and X is the mixed liquor suspended solids concentration.

The F/M ratio ranged from 0.24 to 0.38 per day during Run 5. Generally accepted values for operation of conventional activated sludge processes range from 0.2 to 0.4 per day [32], so the organic loading does not appear to be the cause of the filamentous growth.

F. EFFLUENT SUBSTRATE CONCENTRATION

Runs 1-4

Figure 23 shows a typical chromatogram with phenol and thymol peaks. The effluent substrate concentrations for Runs 1-4 are listed in Tables 21-24 and plotted in Figures 24-27. Concentrations listed as below detection limits (BDL) in the tables are shown as zero on the graphs.

In Run 1, the phenol level increased over the first six hours (during the lag phase of microbial growth) and then began to decrease as microbial activity rose sharply. The effluent concentration continued to fall throughout the remainder of the experiment even though the mixed liquor suspended solids concentration was declining.

The maximum concentration observed in Run 2 was 33 mg/l after one day. The effluent phenol concentration decreased to 6.2 mg/l on Day 5 and remained relatively constant for the remainder of the experiment.

Phenol was not detected in any of the samples collected during Run 3. In Run 4, which was performed under conditions similar to Run 3, the effluent phenol concentrations followed roughly the same pattern exhibited in Run 2.

Problems with the GC arose during several of the analyses. The most frequently encountered problem was clogging of the autosampler. Since most of the concentrations

were only slightly above the detection limit, which was about 1 mg/l, the results should not be taken as precise values. The data can be used to indicate trends.

The most interesting trend in the effluent substrate concentration data is that phenol concentrations continued to decrease or remained stable even though the mixed liquor suspended solids concentration was dropping. This indicates that the metabolic activity of the microorganisms must have increased as the MLSS concentration dropped. The oxygen consumption rate data support this conclusion. As the solids concentration decreased, the OCR remained relatively steady. Therefore, the specific oxygen consumption rate (OCR per amount of MLSS) was increasing.

Run 5

Standard curves for phenol and 2-chlorophenol are presented in Figures 28 - 30. The correlation coefficient for the line which best fit the data was equal to 0.9999 for Figure 28, 0.9997 for Figure 29, and 0.9977 for Figure 30.

Problems with the autoinjector were circumvented by using manual injection. A lower detection limit was obtained with the standard curves, but data near the detection limit are still suspect because that is where the most distortion takes place when linear regression is employed.

The concentration of the influent feed sample was 230 mg/l. This sample, along with the other Run 5 samples,

was stored for almost 90 days before analysis. The result indicates very little reduction in the phenol concentration over time. The 20 mg/l reduction could have been due to biodegradation of the feed while it was in the influent container, as growth was observed in the influent line and solids were occaisionally observed in the influent container.

As a further check on the stability of the substrates, standard solutions of phenol and 2-chlorophenol which were more than nine months old were analyzed and compared to the one-day old standards used to develop the standard curves shown in Figures 28 - 30. The standard which originally contained phenol at 25.0 mg/l was determined to have a phenol concentration of 20.7 mg/l. The 2-chlorophenol standard, originally 20.0 mg/l, had a 2-chlorophenol concentration of 16.9 mg/l.

The effluent substrate concentrations for Run 5 are listed in Table 25 and graphed in Figure 31. Concentrations listed as BDL in Table 25 are shown as zero on the graph. The values for S on Days 23-26 represent 2-chlorophenol concentrations. Phenol was always below detection limits after Day 11. Thus, separation of the two compounds was not a problem. Example chromatograms are shown in Figures 32 and 33.

The substrate degradation rate is calculated according to the equation:

$$R = Q (So - S) / V$$

where Q is the influent flow rate, So is the influent substrate concentration, S is the effluent substrate concentration, and V is the volume of the aeration tank. If a value of 1 mg/l is taken as the average effluent substrate concentration, the average degradation rate is equivalent to 0.68 g/l*day.

Holladay et al. [23] reported values ranging from 1.1 to 2.7 g/l*day for hydraulic detention times ranging from 12.5 to 29.2 hours and influent phenol concentrations of 800 mg/l. The value of 0.68 g/l*day was obtained with a hydraulic detention time of 8.75 hours and an influent substrate concentration of 250 mg/l.

The efficiency of the system (substrate conversion) is determined according to the equation:

$$E = (So - S) / So$$

where So is the influent substrate concentration and S is the effluent substrate concentration. The results demonstrate that the substrate, whether phenol or a combination of phenol and 2-chlorophenol, was removed at better than 99 percent

efficiency on a continuous basis.

Before concluding that substrate removal was entirely due to biodegradation, other potential removal mechanisms must be considered. Other potential removal mechanisms include volatilization (stripping), sorption, photolysis, and metal-catalyzed oxidation [52].

Phenol has a moderately low vapor pressure and a high solubility which implies that there is little tendency for volatilization from water [52]. Previous experiments conducted in our laboratory found stripping to be insignificant during the biodegradation of phenol [17]. Results of studies performed by Rozich et al. [39] are in agreement with this conclusion.

The octanol/water partition coefficient for phenol is 1.46, indicating that it has only a slight tendency to be sorbed onto the biomass [52]. Rozich et al. [39] have found that small amounts of unmetabolized phenol may reside in or on the biomass exiting the system; however, Kincannon et al. [28] found that sorption was not an important mechanism in removing phenol from wastewater.

The presence or absence of light had no influence on the biodegradation of phenol [52]. Hydroxylation of aqueous phenol in the presence of air and iron (III) or copper (II) ions has been reported but at temperatures and pressures far above what would normally be encountered [52].

The vapor pressure of 2-chlorophenol is somewhat higher than that of phenol. However, its high solubility (28,500 mg/l) indicates a relatively low activity coefficient, and therefore a low tendency to volatilize. This has been confirmed by previous studies in our laboratory [14,33].

Sorption and oxidation probably are not significant removal mechanisms, but there is some evidence that photolysis may play a significant role in the degradation of 2-chlorophenol [52].

V. CONCLUSIONS AND COMMENTS

The following conclusions are drawn from the results of these experiments:

1. A bench-scale activated sludge process was operated at a relative steady state using phenol and a combination of phenol and 2-chlorophenol as the sole carbon source.
2. Phenol and 2-chlorophenol were removed at better than 99 percent efficiency on a continuous basis.
3. The mixed liquor grown on phenol under the conditions of the experiment was highly filamentous and exhibited poor settling characteristics.
4. The process can be successfully operated with a predominantly filamentous culture provided the clarifier is not overloaded and the sludge blanket does not escape in the effluent.

It is suggested that further research be conducted to determine the conditions which lead to filamentous growth in mixed liquor grown on phenol. It is also suggested that a more sensitive analytical procedure be employed to determine effluent phenol and 2-chlorophenol concentrations at the microgram per liter (part per billion) level.

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TABLES

TABLE 1

ELEMENTAL CELL COMPOSITION

Element	Dry Weight Percent
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorus	3
Sulfur	1
Potassium	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
All Others	0.3

Source: Gaudy, A. F., et al., Microbiology for Environmental Scientists and Engineers. New York, NY: McGraw-Hill, 1980.

TABLE 2

HYDRAULIC AND MIXING DATA

TIME (min)	CONC (mg/l)	t/T	Percent Recovery
0	0	0	0
0.5	0	0.003	0.3
1	0	0.006	0.6
2	0	0.013	1.3
4	0.71	0.025	2.5
8	0.91	0.050	4.9
12	0.92	0.075	7.2
16	0.87	0.10	9.5
24	0.86	0.15	13.5
36	0.81	0.23	20.5
48	0.74	0.30	25.9
60	0.68	0.38	31.6
72	0.63	0.45	36.2
84	0.59	0.53	41.1
96	0.53	0.60	45.1
108	0.49	0.68	49.3
120	0.46	0.75	52.8
132	0.43	0.83	56.4
144	0.39	0.91	59.7
156	0.36	0.98	62.5
168	0.33	1.06	65.4
180	0.31	1.13	67.7
192	0.28	1.21	70.2
204	0.26	1.28	72.2
216	0.26	1.36	74.3
228	0.25	1.43	76.1
240	0.21	1.51	77.9

TABLE 3

OXYGEN TRANSFER
TAP WATER

TIME (min)	DO (mg/l)
0	0.18
0.5	1.38
1.0	2.64
1.5	3.52
2.0	4.47
2.5	5.30
3.0	5.92
3.5	6.42
4.0	6.93
4.5	7.26
5.0	7.54
5.5	7.68
6.0	8.01
6.5	8.12
7.0	8.25
7.5	8.43
8.0	8.58
8.5	8.66
9.0	8.72
9.5	8.78
10.0	8.84
10.5	8.86
11.0	8.90
11.5	8.92
13.0	8.99
13.5	9.00
14.0	9.04
14.5	9.04
15.0	9.04

TABLE 4

OXYGEN TRANSFER
MIXED LIQUOR

TIME (min)	DO (mg/l)	TIME (min)	DO (mg/l)
0	0.17	18.5	6.06
0.5	0.36	19.0	6.13
1.0	0.46	19.5	6.21
1.5	0.53	20.0	6.27
2.0	0.61	20.5	6.36
2.5	0.69	21.0	6.49
3.0	0.77	21.5	6.55
3.5	0.88	22.0	6.61
4.0	0.93	22.5	6.65
4.5	1.08	23.0	6.74
5.0	1.24	23.5	6.83
5.5	1.43	24.0	6.89
6.0	1.66	24.5	6.92
6.5	1.81	25.0	6.98
7.0	2.15	25.5	7.04
7.5	2.43	26.0	7.07
8.0	2.59	26.5	7.08
8.5	2.82	27.0	7.10
9.0	3.03	27.5	7.13
9.5	3.25	28.0	7.18
10.0	3.48	28.5	7.21
10.5	3.75	29.0	7.23
11.0	3.92	29.5	7.26
11.5	4.13	30.0	7.29
12.0	4.33	31.0	7.34
12.5	4.52	32.0	7.39
13.0	4.71	33.0	7.46
13.5	4.85	34.0	7.50
14.0	4.98	35.0	7.54
14.5	5.11	36.0	7.61
15.0	5.30	37.0	7.66
15.5	5.44	38.0	7.70
16.0	5.56	39.0	7.79
16.5	5.62	40.0	7.78
17.0	5.77	45.0	7.80
17.5	5.89	50.0	7.80
18.0	5.97		

TABLE 5

TEMPERATURE AND pH
RUN 1

TIME (hours)	T (C)	pH (su)
0	25	7.7
1	25	7.7
2	26	7.6
3	26	7.6
6	26	7.5
12	24	7.3
24	26	7.0
36	23	7.0
48	25	7.0
60	24	6.8

TABLE 6

TEMPERATURE AND pH
RUN 2

TIME (days)	T (C)	pH (su)
0	25	7.6
1	25	7.4
2	25	7.0
3	28	6.8
4	29	6.9
5	26	6.8
6	25	6.5
7	25	6.8
8	24	6.8
9	23	6.8
10	23	6.6
11	24	6.7
12	23	6.7

TABLE 7

TEMPERATURE AND pH
RUN 3

TIME (days)	T (C)	pH (su)
0	24	6.6
1	24	6.5
2	25	6.9
3	25	6.8
4	25	6.8
5	25	6.9
6	25	6.8
7	25	6.8
8	25	6.8
9	23	6.8
10	28	6.8
11	25	6.8

TABLE 8

TEMPERATURE AND pH
RUN 4

TIME (days)	T (C)	pH (su)
0	20	6.9
1	19	6.8
2	21	6.8
3	19	6.8
4	19	6.8
5	19	6.9
6	21	6.8
7	22	6.8
8	22	6.7
9	21	6.9

TABLE 9

TEMPERATURE AND pH
RUN 5

TIME (days)	T (C)	pH (su)
0	23	7.9
1	23	7.6
2	22	7.5
3	22	7.5
4	23	7.6
5	23	7.4
6	24	7.3
7	23	7.1
8	24	7.1
9	24	7.1
10	25	7.1
11	25	7.0
12	24	6.9
13	24	7.1
14	23	7.1
15	23	7.1
16	23	7.1
17	24	7.1
18	24	7.2
19	24	7.1
20	23	7.1
21	23	7.1
22	24	7.2
23	24	7.1
24	24	7.0
25	25	7.0
26	24	6.9
27	24	7.0
28	25	7.0
29	24	6.9
30	25	7.0
31	24	6.9

TABLE 10

DISSOLVED OXYGEN AND
OXYGEN CONSUMPTION RATE
RUN 1

TIME (hours)	DO (mg/l)	OCR (mg/l/min)
0	7.5	0.18
1	7.6	0.20
2	7.5	0.22
3	7.4	0.21
6	7.4	0.29
12	6.8	1.04
24	6.9	0.99
36	7.0	1.03
48	6.9	0.86
60	7.6	0

TABLE 11

DISSOLVED OXYGEN AND
OXYGEN CONSUMPTION RATE
RUN 2

TIME (days)	DO (mg/l)	OCR (mg/l/min)
0	6.5	0.26
1	5.8	1.08
2	5.7	1.00
3	5.6	0.94
4	5.3	0.88
5	5.0	0.97
6	5.2	0.91
7	5.3	0.95
8	5.2	0.89
9	5.6	0.89
10	5.6	0.94
11	5.9	0.85
12	6.0	0.82

TABLE 12

DISSOLVED OXYGEN AND
OXYGEN CONSUMPTION RATE
RUN 3

TIME (days)	DO (mg/l)	OCR (mg/l/min)
0	5.5	0.65
1	5.2	0.90
2	4.7	0.99
3	4.9	1.29
4	4.7	1.09
5	4.7	0.90
6	5.2	0.96
7	4.9	0.99
8	4.9	0.94
9	4.7	1.02
10	5.0	1.01
11	5.3	0.98

TABLE 13

DISSOLVED OXYGEN AND
OXYGEN CONSUMPTION RATE
RUN 4

TIME (days)	DO (mg/l)	OCR (mg/l/min)
0	6.2	0.30
1	5.7	0.73
2	5.8	1.12
3	5.4	1.38
4	5.4	1.20
5	5.3	1.23
6	5.5	1.35
7	5.5	1.15
8	5.6	0.87
9	8.0	0

TABLE 14

DISSOLVED OXYGEN AND
OXYGEN CONSUMPTION RATE
RUN 5

TIME (days)	DO (mg/l)	OCR (mg/l/min)
0	6.4	0.45
1	5.8	0.98
2	5.8	0.85
3	5.6	0.66
4	5.8	0.72
5	5.9	0.32
6	5.8	0.28
7	5.4	0.27
8	5.6	0.29
9	5.8	0.70
10	5.5	0.26
11	5.7	0.68
12	5.7	0.35
13	5.7	0.40
14	5.5	0.56
15	5.6	0.65
16	5.8	0.34
17	5.9	0.29
18	5.8	0.40
19	6.0	0.62
20	5.7	0.43
21	5.5	0.35
22	5.8	0.29
23	5.7	0.30
24	6.0	0.31
25	5.9	0.28
26	5.6	0.24
27	5.5	0.26
28	5.5	0.27
29	5.8	0.31
30	5.6	0.31
31	5.9	0.29

TABLE 15

SUSPENDED SOLIDS
RUN 1

TIME (hours)	MLSS (mg/l)
0	2800
12	2500
24	2200
36	1500
48	1100
60	700

TABLE 16

SUSPENDED SOLIDS
RUN 2

TIME (days)	MLSS (mg/l)
0	3000
1	3300
2	3100
3	2800
4	2400
5	2100
6	1900
7	1700
8	1600
9	1300
10	1100
11	900
12	600

TABLE 17

SUSPENDED SOLIDS
RUN 3

TIME (days)	AERATION TANK (mg/l)	SLUDGE RECYCLE (mg/l)	EFFLUENT (mg/l)
0	3000	-	-
1	2800	5600	300
2	3000	7200	100
3	3500	6500	200
4	3100	6100	300
5	2600	4900	1000
6	2200	4200	700
7	2000	3500	600
8	1700	2500	800
9	1200	1900	700
10	1000	1500	700
11	600	1100	900

TABLE 18

SUSPENDED SOLIDS
RUN 4

TIME (days)	AERATION TANK (mg/l)	SLUDGE RECYCLE (mg/l)	EFFLUENT (mg/l)
0	2500	-	-
1	2500	5500	300
2	2800	4600	200
3	3100	4900	300
4	2700	4200	300
5	2800	4500	200
6	2700	4200	700
7	1800	3000	700
8	1500	2800	900
9	700	1200	1100

TABLE 19

SUSPENDED SOLIDS
RUN 5

TIME (days)	AERATION TANK (mg/l)	SLUDGE RECYCLE (mg/l)	EFFLUENT (mg/l)
0	2100	-	-
1	2000	-	-
2	2100	5900	<100
3	2000	-	-
4	1800	4900	<100
5	1800	-	-
6	1900	4600	<100
7	1800	-	-
8	2000	4700	<100
9	2000	-	-
10	2400	3400	<100
11	2300	-	-
12	2500	3000	<100
13	2700	-	-
14	2800	3200	<100
15	2600	-	-
16	2600	2800	<100
17	2500	-	-
18	2600	3100	100
19	2500	-	-
20	2400	3000	<100
21	2300	-	-
22	2400	3200	<100
23	2400	-	-
24	2200	3200	200
25	2300	-	-
26	2200	3200	100
27	2000	-	-
28	2400	3000	<100
29	2500	-	-
30	2300	2900	100
31	2400	-	-

TABLE 20

PREDOMINANT MICROBIAL AND FUNGAL GENERA IN MIXED LIQUOR

Bacteria:	Gram positive/Gram negative = 1/10
	1. Acinetobactor anitratus
	2. Acinetobacter lwoffii
	3. Enterobacter agglomerans
	4. Enterobacter cloacae
	5. Group 2k-1 (Pseudomonas-like)
	6. Proteus vulgaris
	7. Providencia stuartii
	8. Pseudomonas cepacia
	9. Pseudomonas maltophilia
	10. Serratia marcescens
Yeasts:	1. Trichosporon beigleii
	2. C. albicans
	3. C. stellatoidea
Molds:	1. Penicillum spp
	2. Aspergillus flavus
	3. Aspergillus niger
	4. Trichophyta spp (tonsurans or violaceum)
Protists:	1. Coleps spp
	2. Uniflagellate (unidentified)

TABLE 21

EFFLUENT SUBSTRATE CONCENTRATION
RUN 1

TIME (hours)	S (mg/l)
0	BDL
1	5.2
2	30
3	48
6	162
12	150
24	67
36	19
48	2.0
60	BDL

NOTE: BDL = Below Detection Limit

TABLE 22

EFFLUENT SUBSTRATE CONCENTRATION
RUN 2

TIME (days)	S (mg/l)
0	BDL
1	33
2	17
3	20
4	11
5	6.2
6	5.2
7	4.7
8	5.5
9	4.9
10	BDL
11	4.2
12	BDL

NOTE: BDL = Below Detection Limit

TABLE 23

EFFLUENT SUBSTRATE CONCENTRATION
RUN 3

TIME (days)	S (mg/l)
0	BDL
1	BDL
2	BDL
3	BDL
4	BDL
5	BDL
6	BDL
7	BDL
8	BDL
9	BDL
10	BDL
11	BDL

NOTE: BDL = Below Detection Limit

TABLE 24

EFFLUENT SUBSTRATE CONCENTRATION
RUN 4

TIME (days)	S (mg/l)
0	BDL
1	19
2	5.9
3	4.5
4	4.0
5	4.3
6	BDL
7	4.9
8	BDL
9	BDL

NOTE: BDL = Below Detection Limit

TABLE 25

EFFLUENT SUBSTRATE CONCENTRATION
RUN 5

TIME (days)	S (mg/l)
0	BDL
1	1.8
2	0.98
3	0.63
4	BDL
5	0.49
6	BDL
7	BDL
8	BDL
9	0.56
10	BDL
11	0.57
12	BDL
13	BDL
14	BDL
15	BDL
16	BDL
17	BDL
18	BDL
19	BDL
20	BDL
21	BDL
22	BDL
23	1.3
24	4.6
25	0.48
26	0.38
27	BDL
28	BDL
29	BDL
30	BDL
31	BDL

NOTE: BDL = Below Detection Limit

FIGURES

FIGURE 1

BIO-OXIDATION APPARATUS

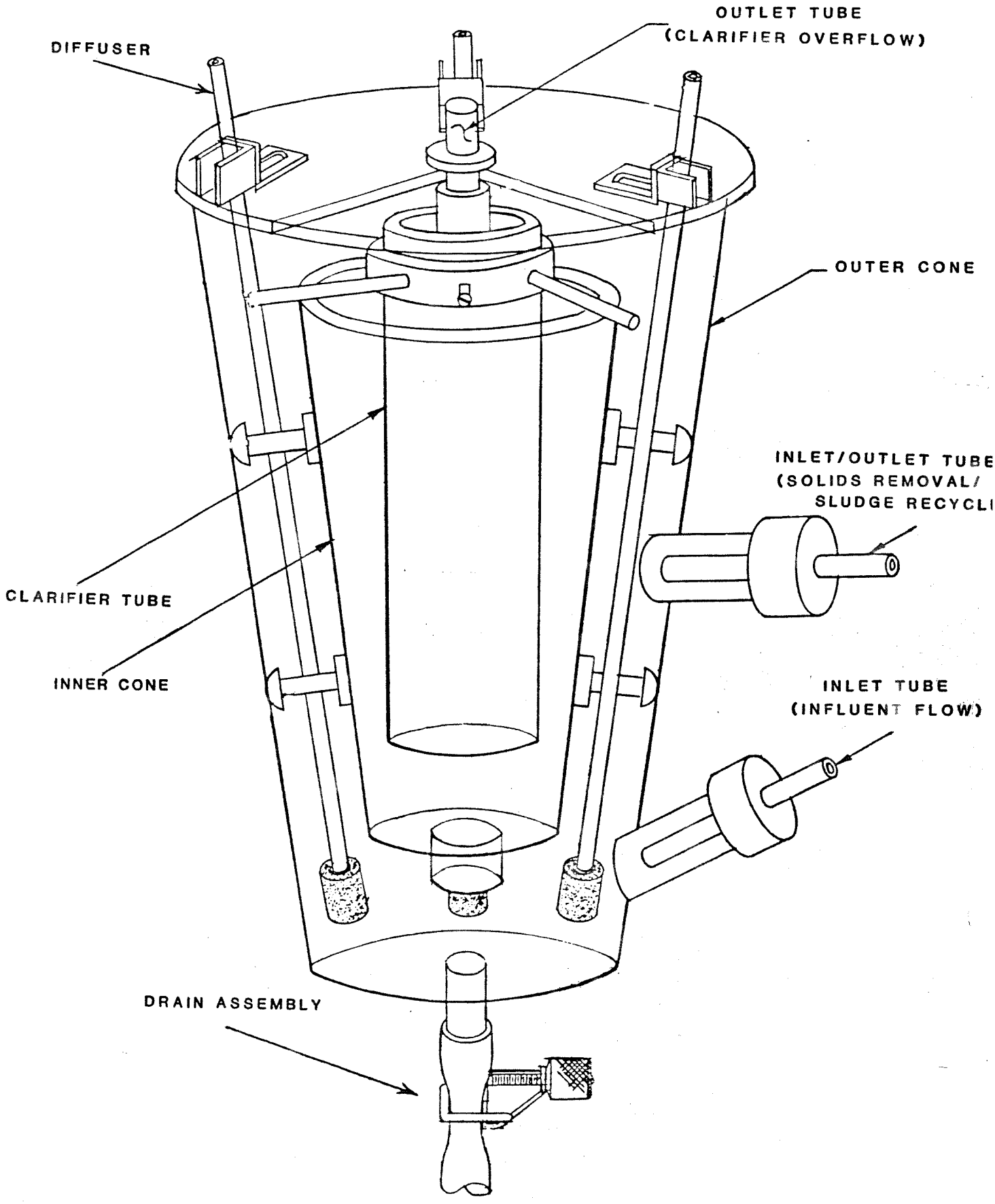
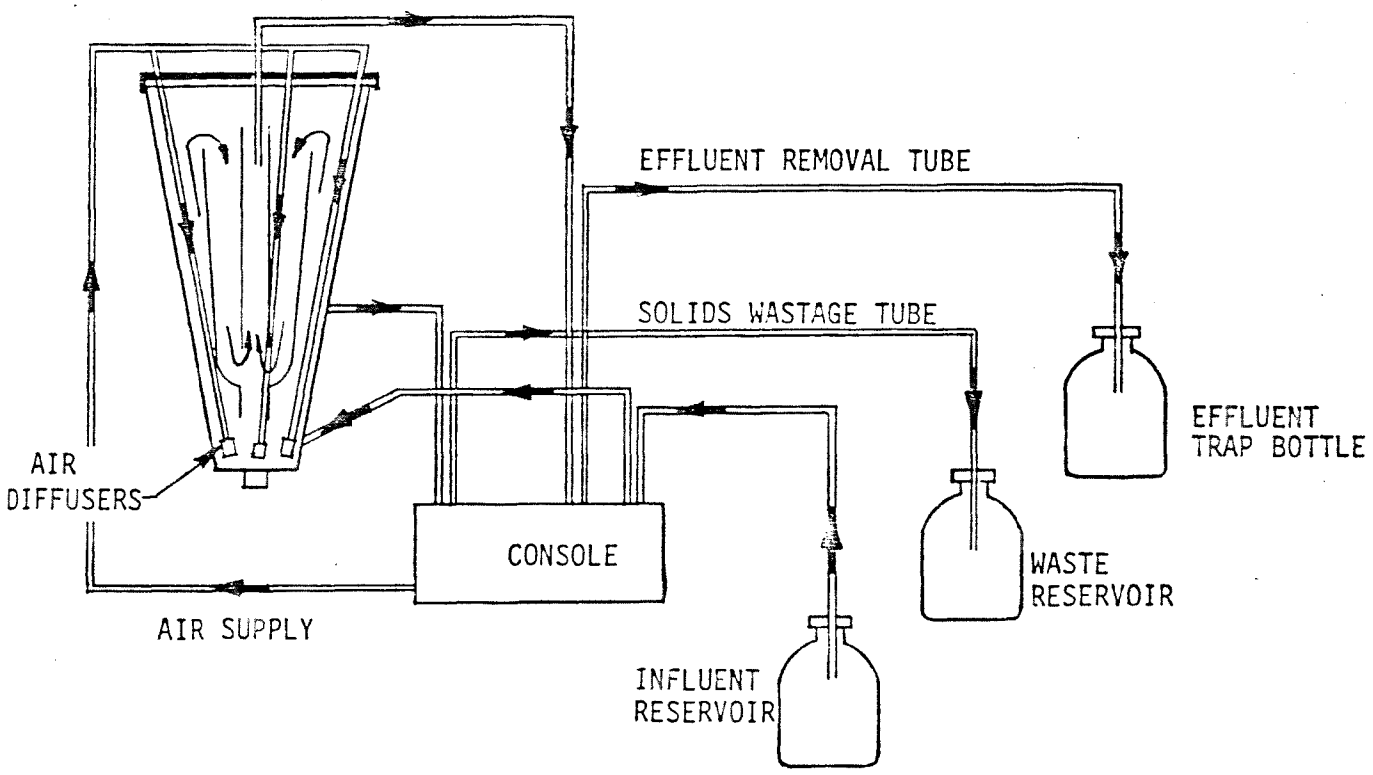


FIGURE 2

FLOW PATTERN IN SYSTEM



ARROWS SHOW DIRECTION OF AIR AND LIQUID FLOW

FIGURE 3

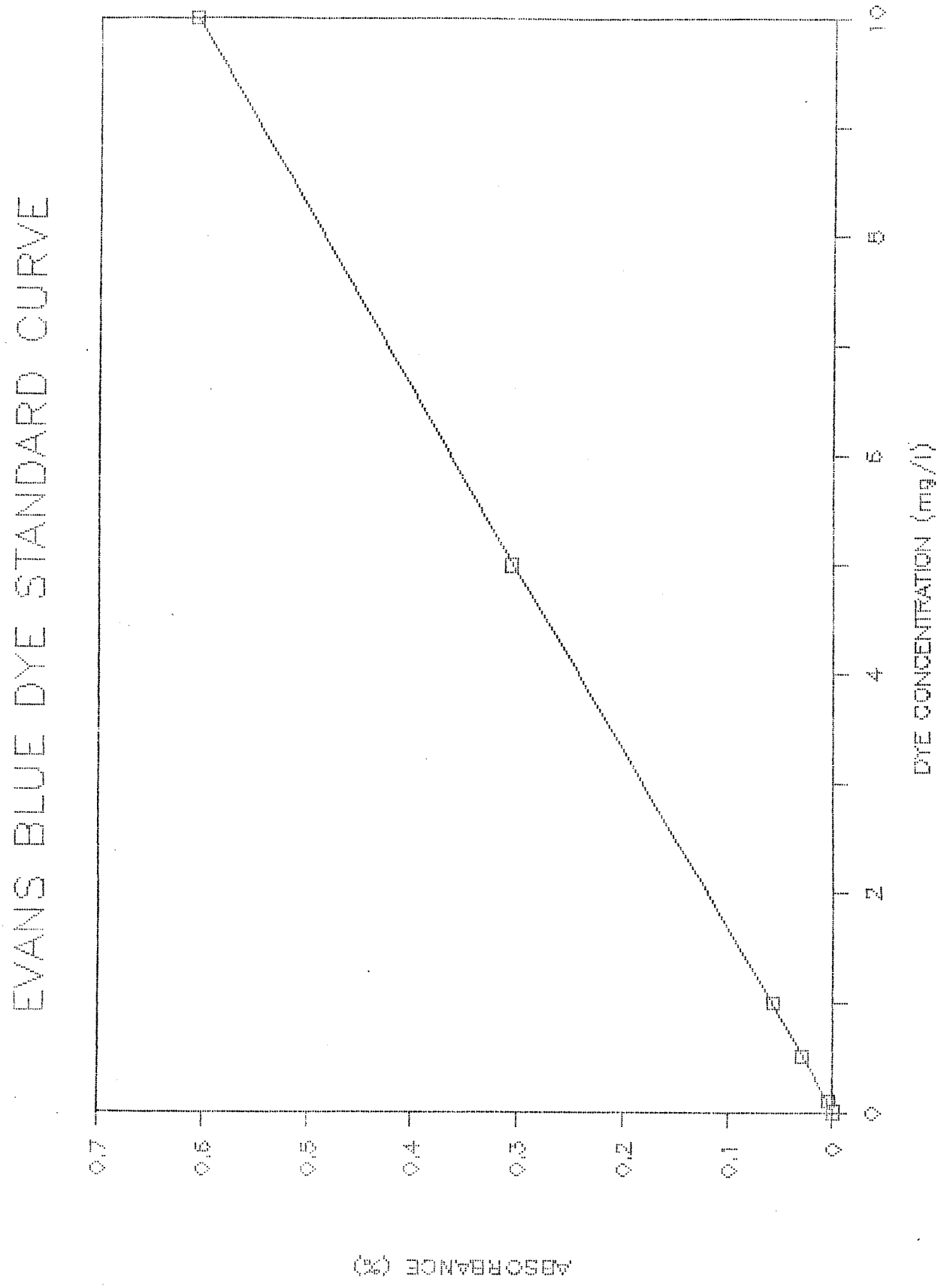

EVANS BLUE DYE STANDARD CURVE
ABSORBANCE (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
DYE CONCENTRATION (mg/l)

FIGURE 4

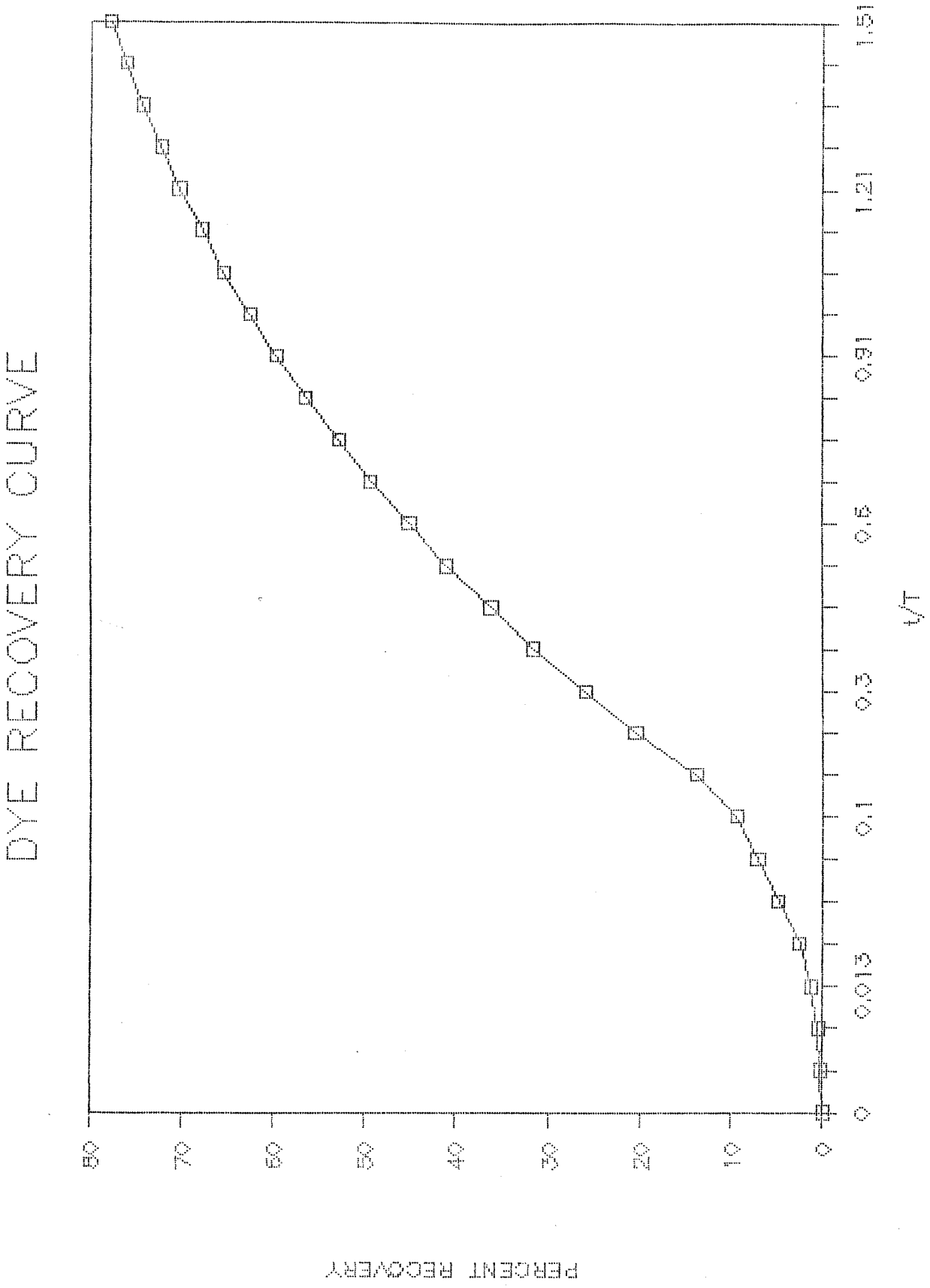

DYE RECOVERY CURVE
PERCENT RECOVERY
80
70
60
50
40
30
20
10
0
0
0.013
0.1
0.3
0.6
0.91
1.21
1.51
t/T

FIGURE 5

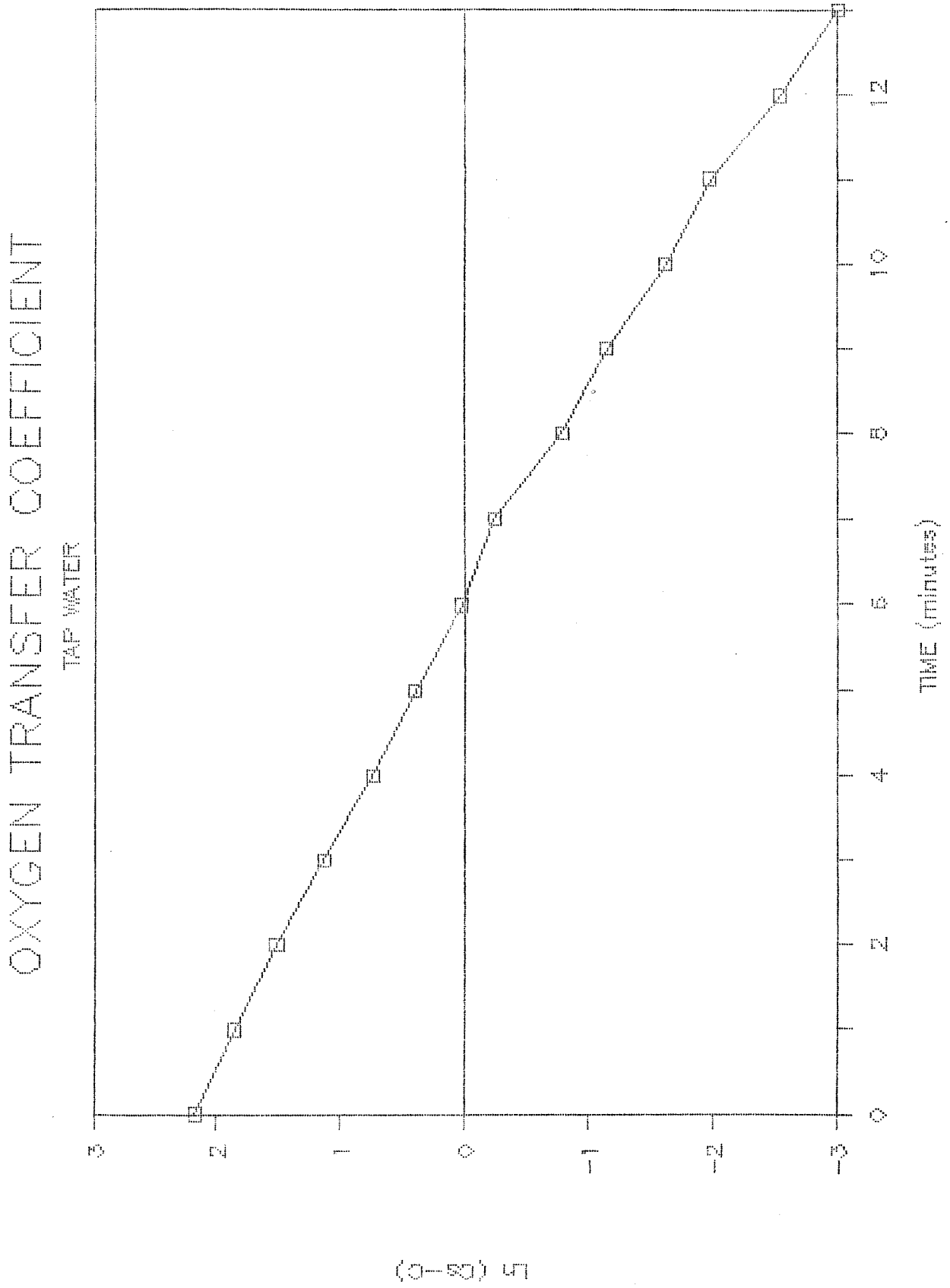

OXYGEN TRANSFER COEFFICIENT
TAP WATER
TIME (minutes)
0
2
4
6
8
10
12
-3
-2
-1
0
1
2
3
(0-50) 57

FIGURE 6

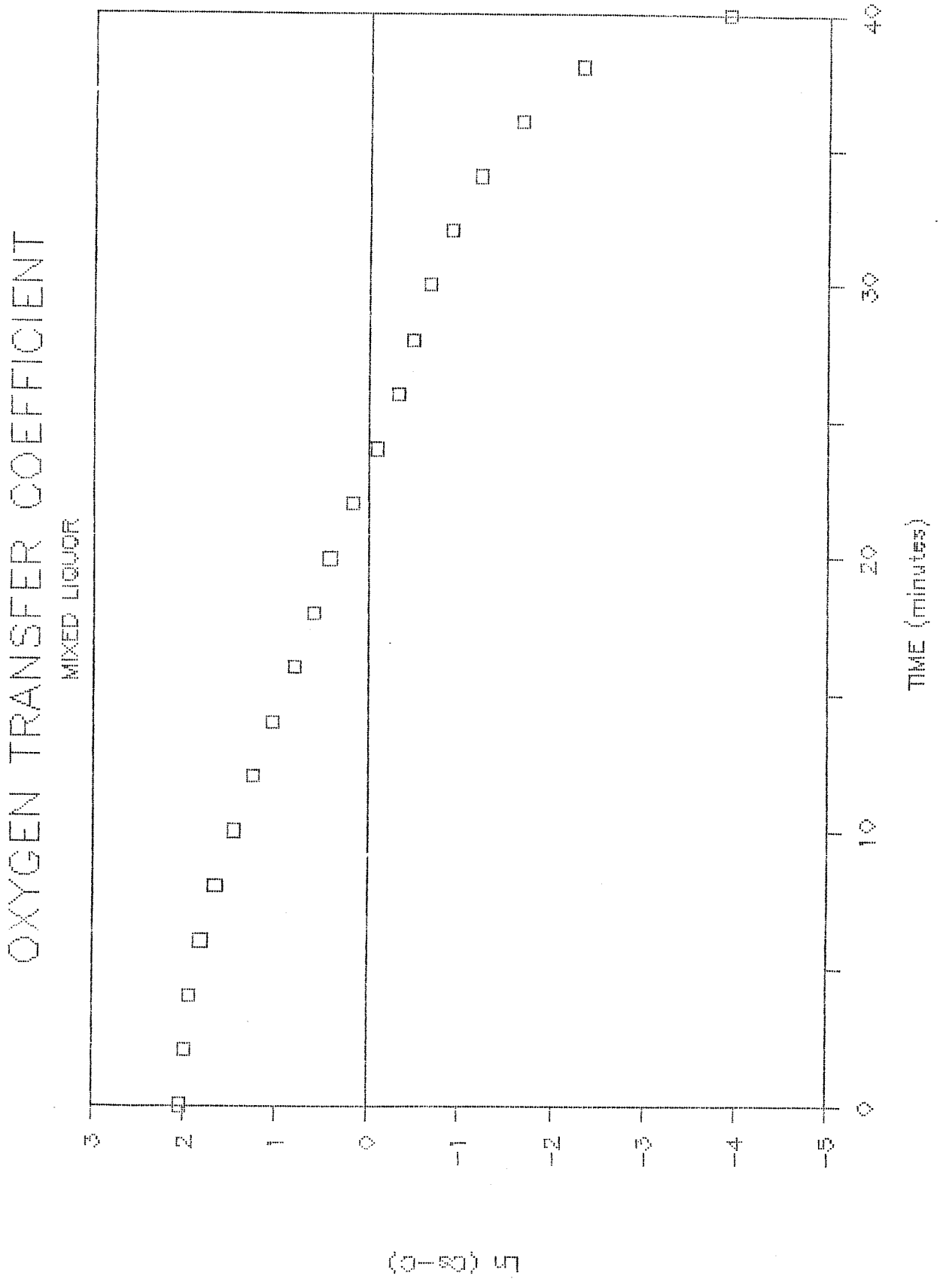

OXYGEN TRANSFER COEFFICIENT
MIXED LIQUOR
TIME (minutes)
0
10
20
30
40
-3
-2
-1
0
1
2
3
4
5
ln (Cs-C)

FIGURE 7

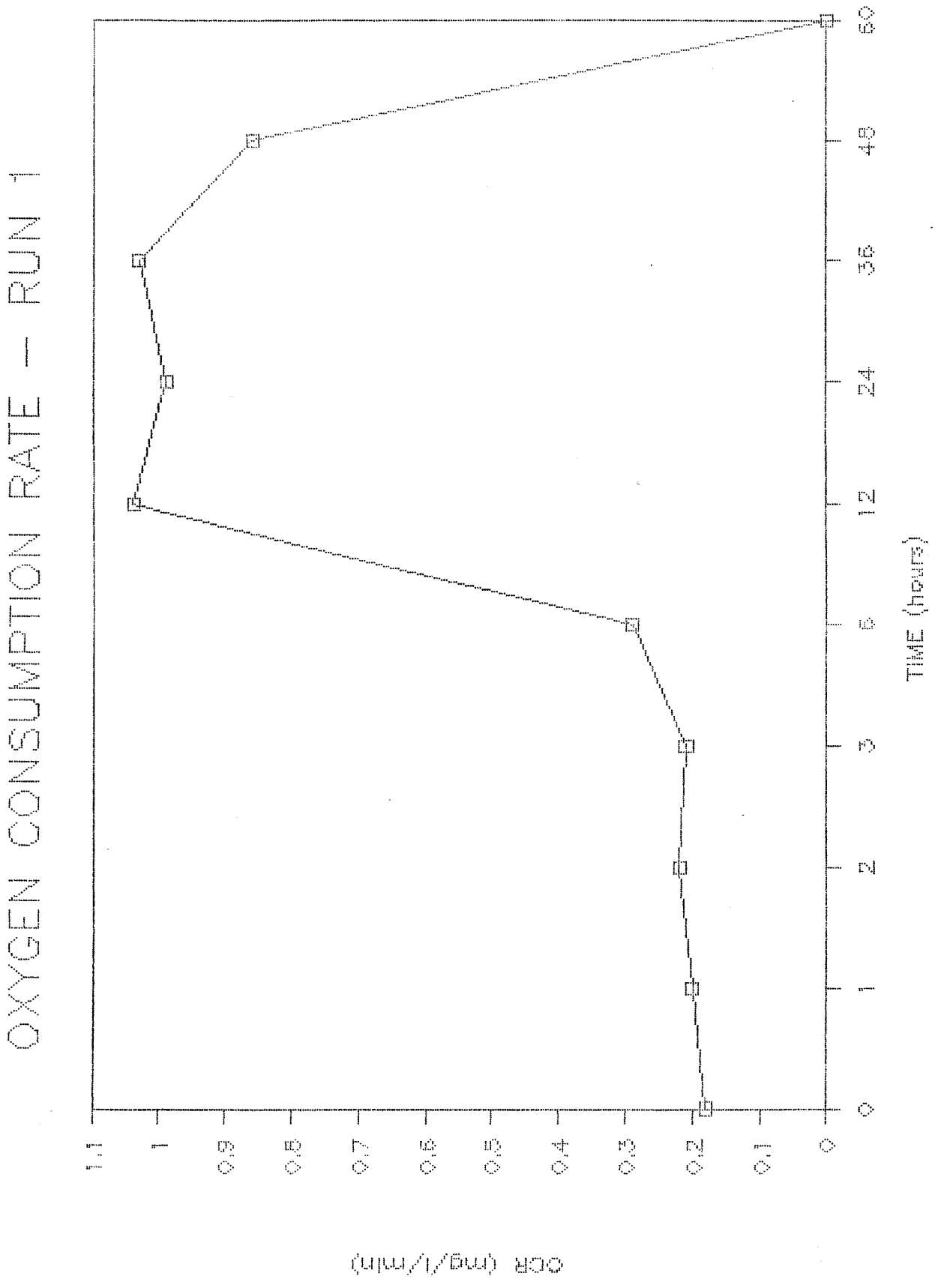

OXYGEN CONSUMPTION RATE - RUN 1
OCR (mg/l/min)
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
6
12
24
36
48
60
TIME (hours)

FIGURE 8

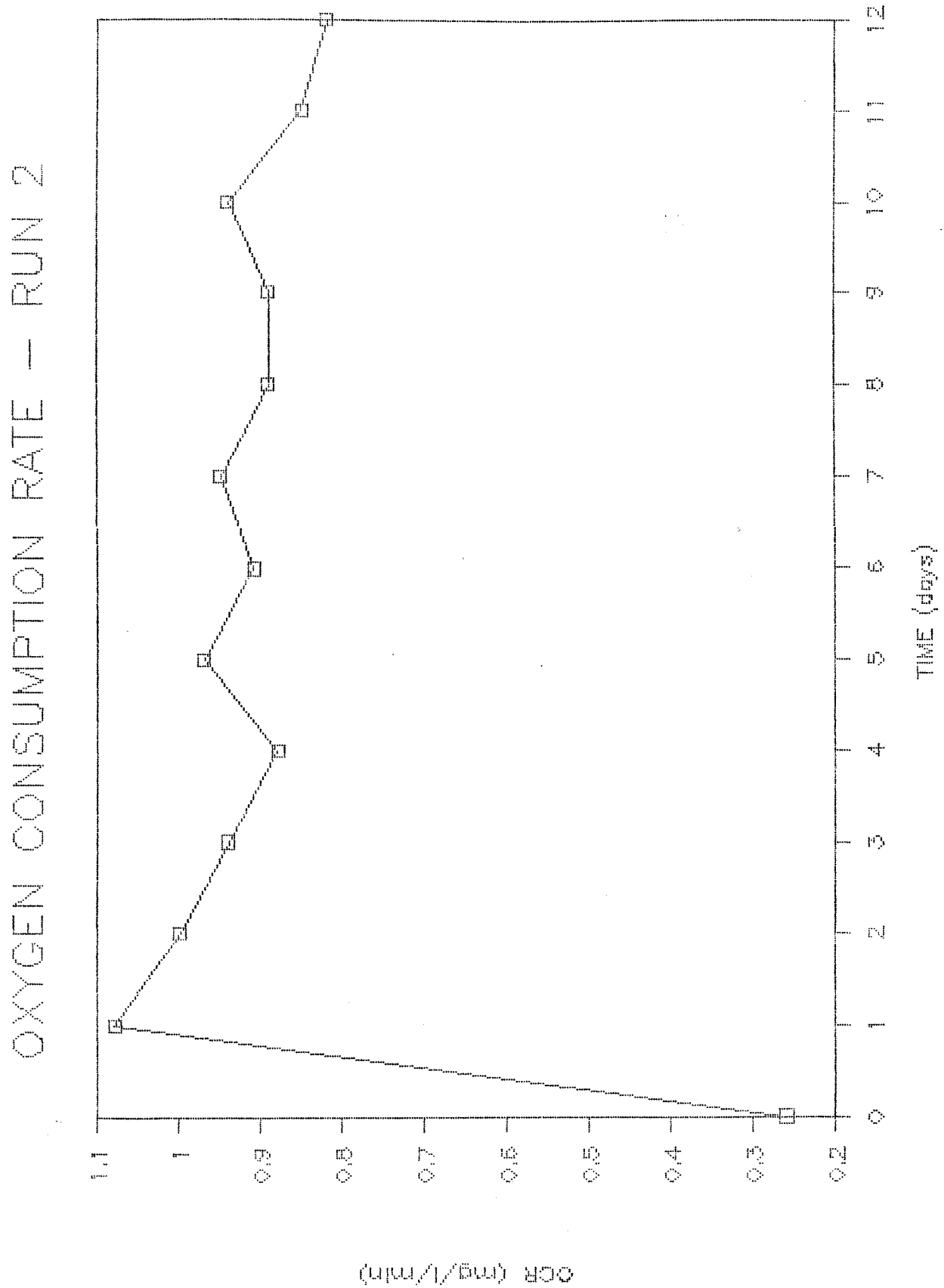

OXYGEN CONSUMPTION RATE — RUN 2
OCR (mg/l/min)
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
1
2
3
4
5
6
7
8
9
10
11
12
TIME (days)

FIGURE 9

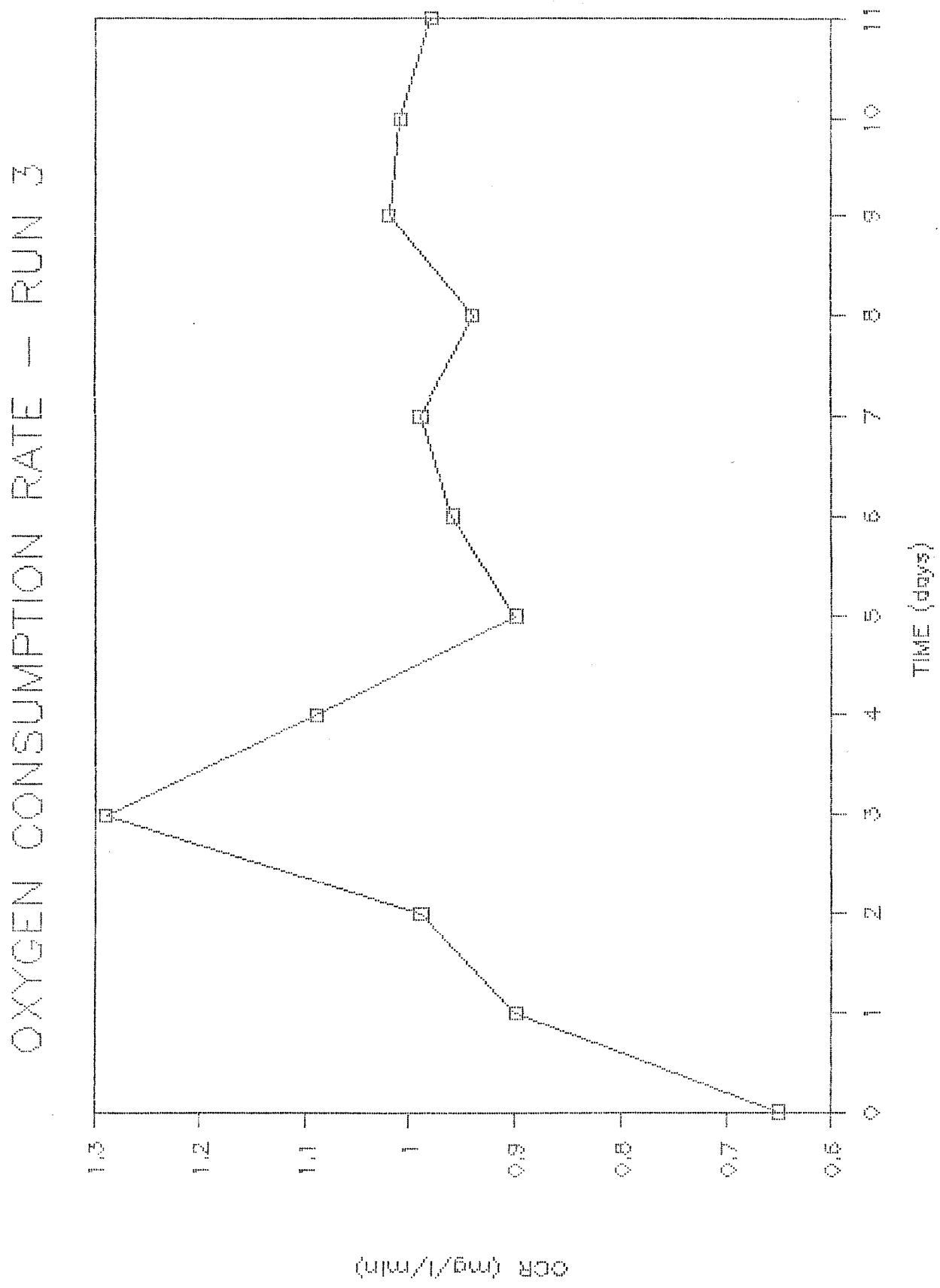

OXYGEN CONSUMPTION RATE — RUN 3
OCR (mg/l/min)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0
1
2
3
4
5
6
7
8
9
10
11
TIME (days)

FIGURE 10

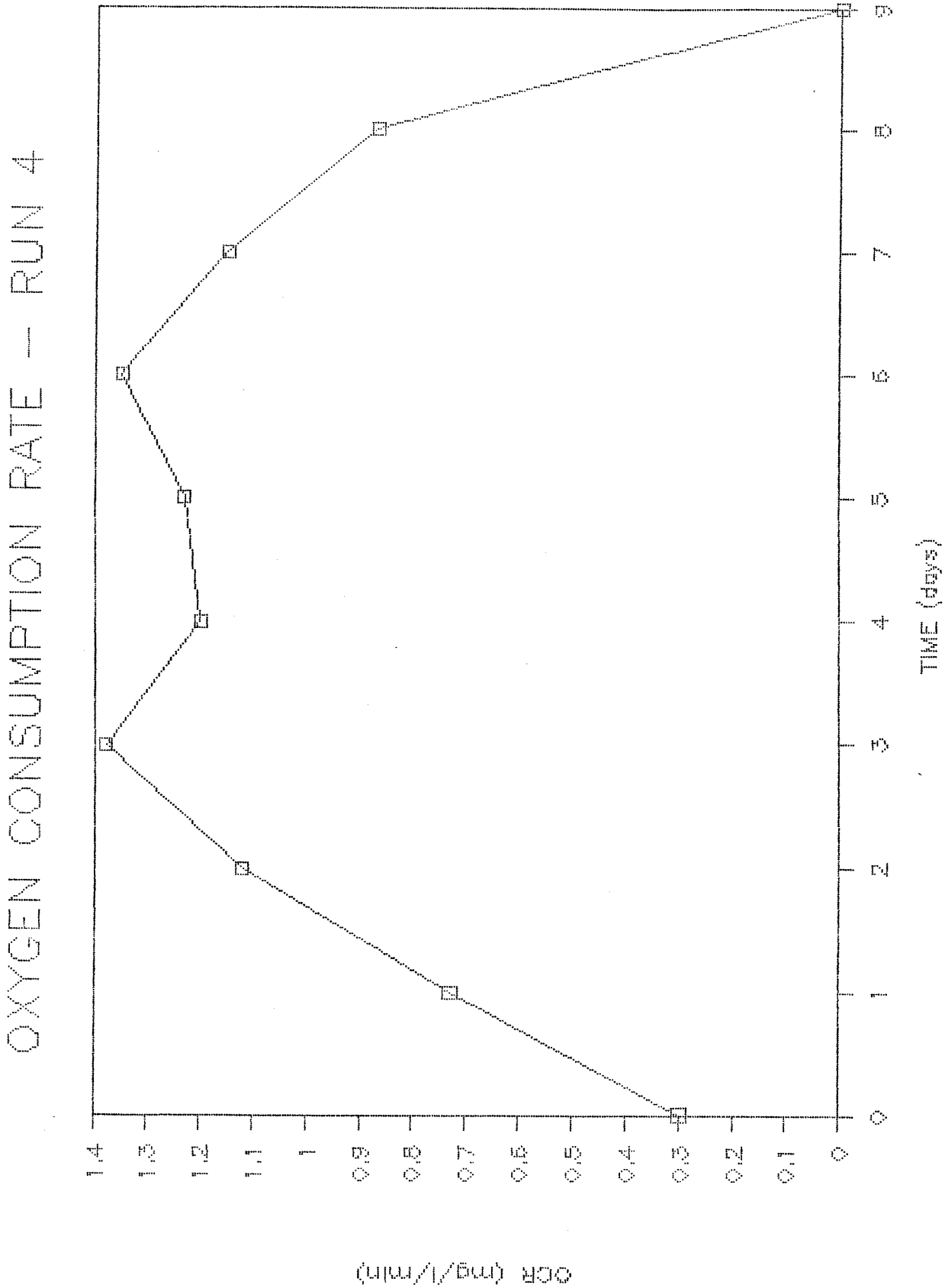

OXYGEN CONSUMPTION RATE — RUN 4
OCR (mg/l/min)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
TIME (days)

FIGURE 11

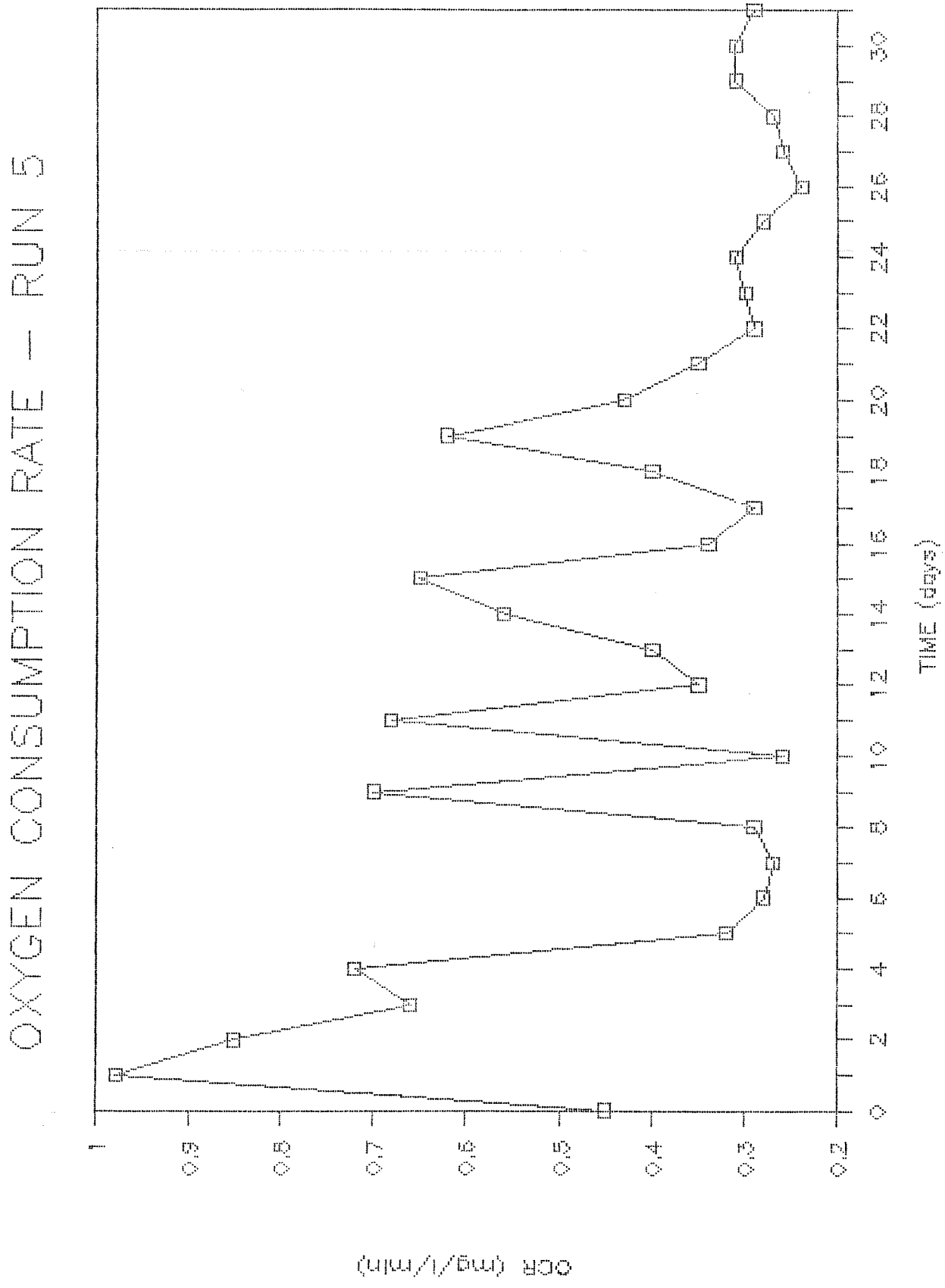

OXYGEN CONSUMPTION RATE — RUN 5
OCR (mg/l/min)
TIME (days)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30

FIGURE 12

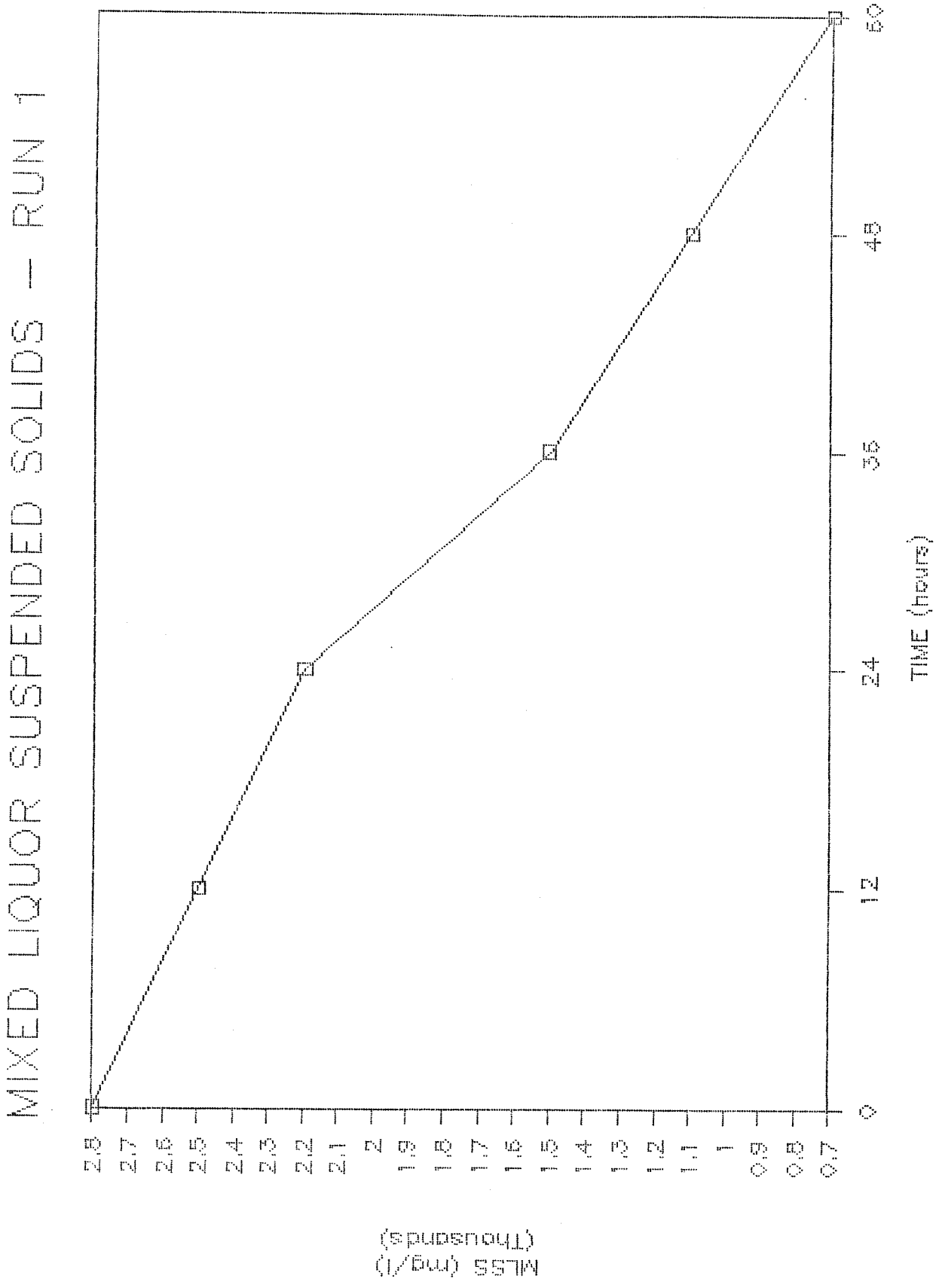

MIXED LIQUOR SUSPENDED SOLIDS - RUN 1
MLSS (mg/l)
(Thousands)
2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1 0.9 0.8 0.7
0 12 24 36 48 60
TIME (hours)

FIGURE 13

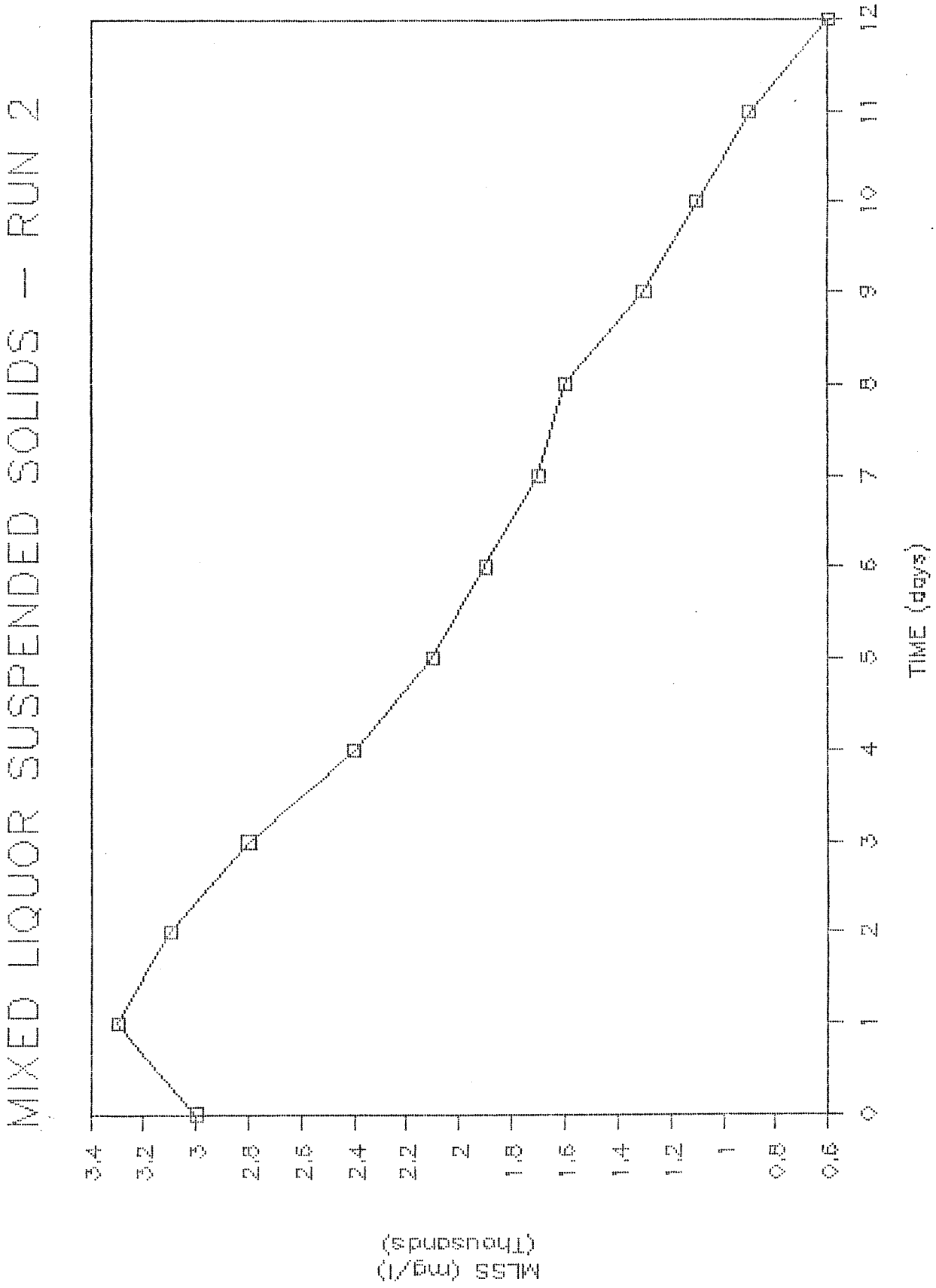

MIXED LIQUOR SUSPENDED SOLIDS - RUN 2
MLSS (mg/l)
(Thousands)
TIME (days)
0
1
2
3
4
5
6
7
8
9
10
11
12
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4
2.6
2.8
3
3.2
3.4

FIGURE 14

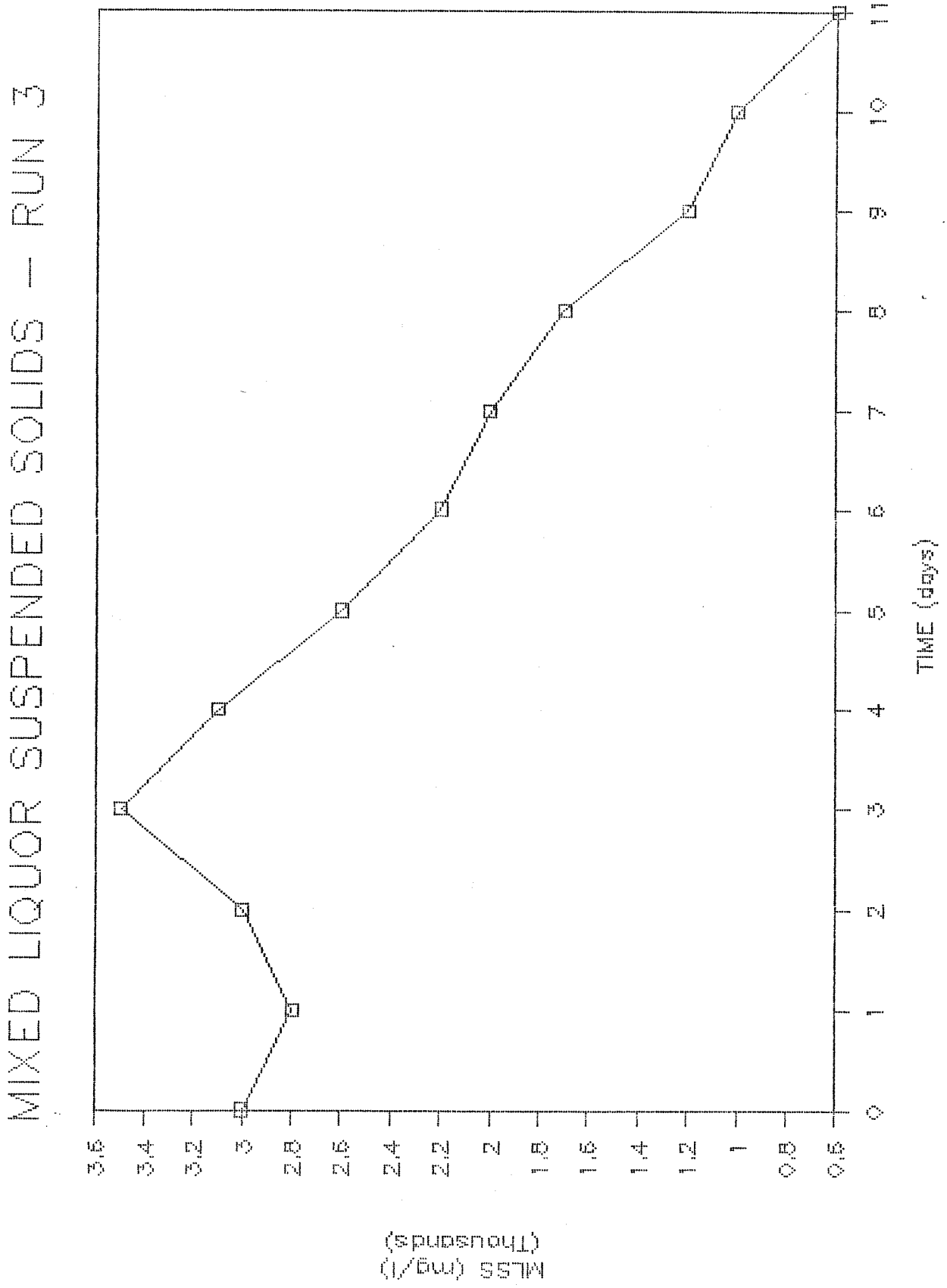

MIXED LIQUOR SUSPENDED SOLIDS - RUN 3
MLSS (mg/l)
(Thousands)
3.6
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0
1
2
3
4
5
6
7
8
9
10
11
TIME (days)

FIGURE 15

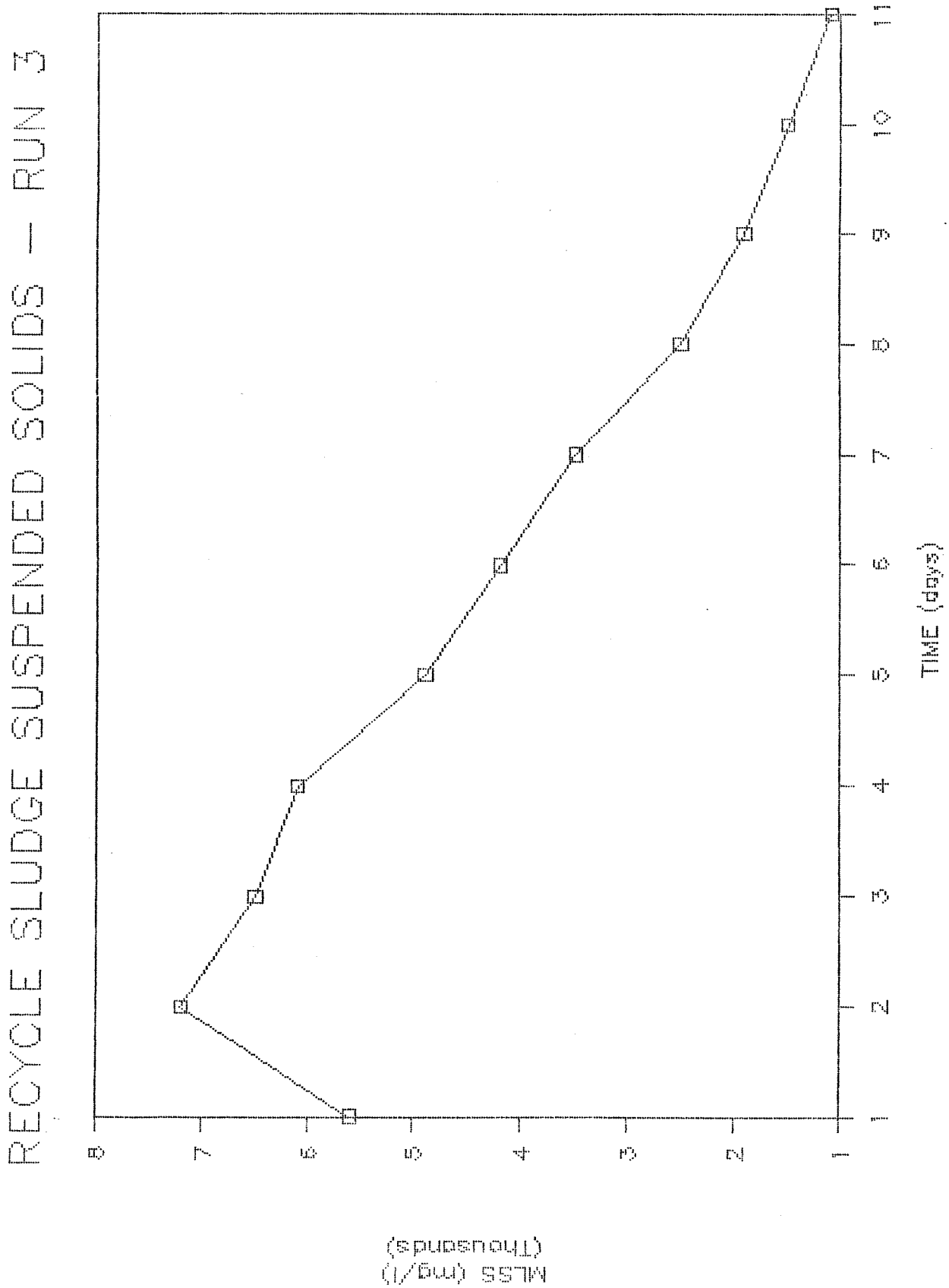

RECYCLE SLUDGE SUSPENDED SOLIDS — RUN 3
MLSS (mg/l)
(Thousands)
8
7
6
5
4
3
2
1
1
2
3
4
5
6
7
8
9
10
11
TIME (days)

FIGURE 16

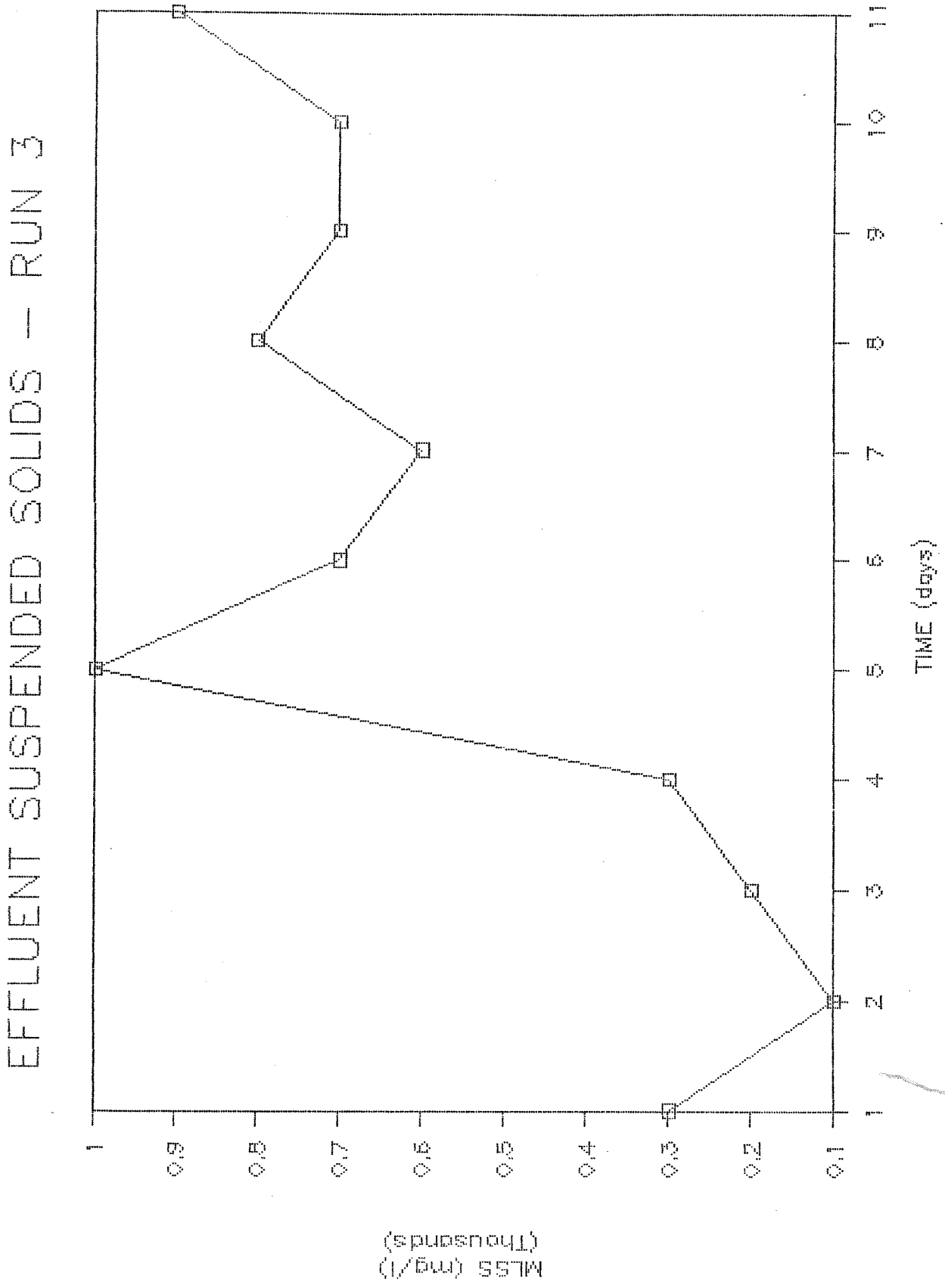

EFFLUENT SUSPENDED SOLIDS — RUN 3
MLSS (mg/l)
(Thousands)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1
2
3
4
5
6
7
8
9
10
11
TIME (days)

FIGURE 17

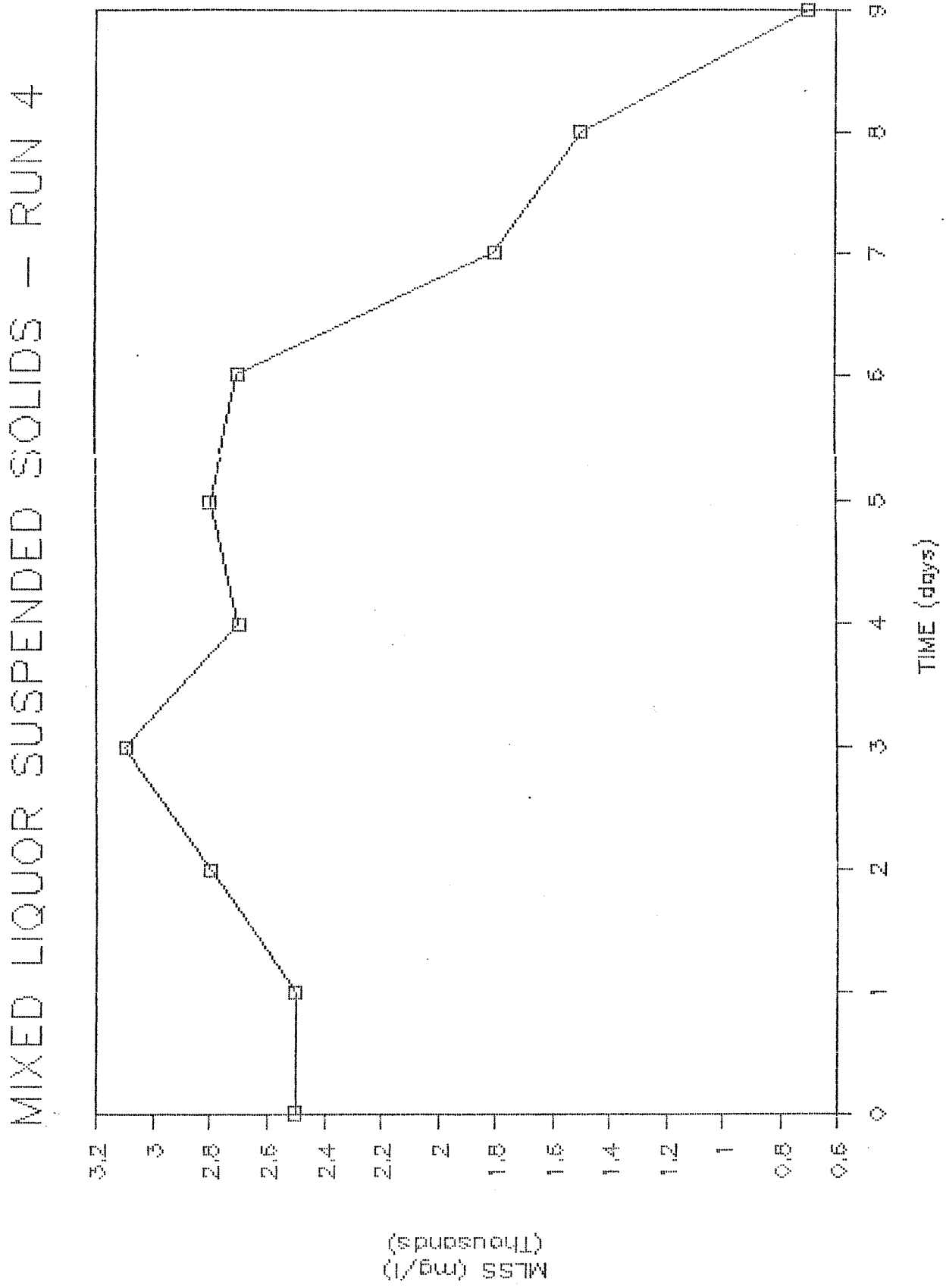

MIXED LIQUOR SUSPENDED SOLIDS — RUN 4
MLSS (mg/l)
(Thousands)
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0
1
2
3
4
5
6
7
8
9
TIME (days)

FIGURE 18

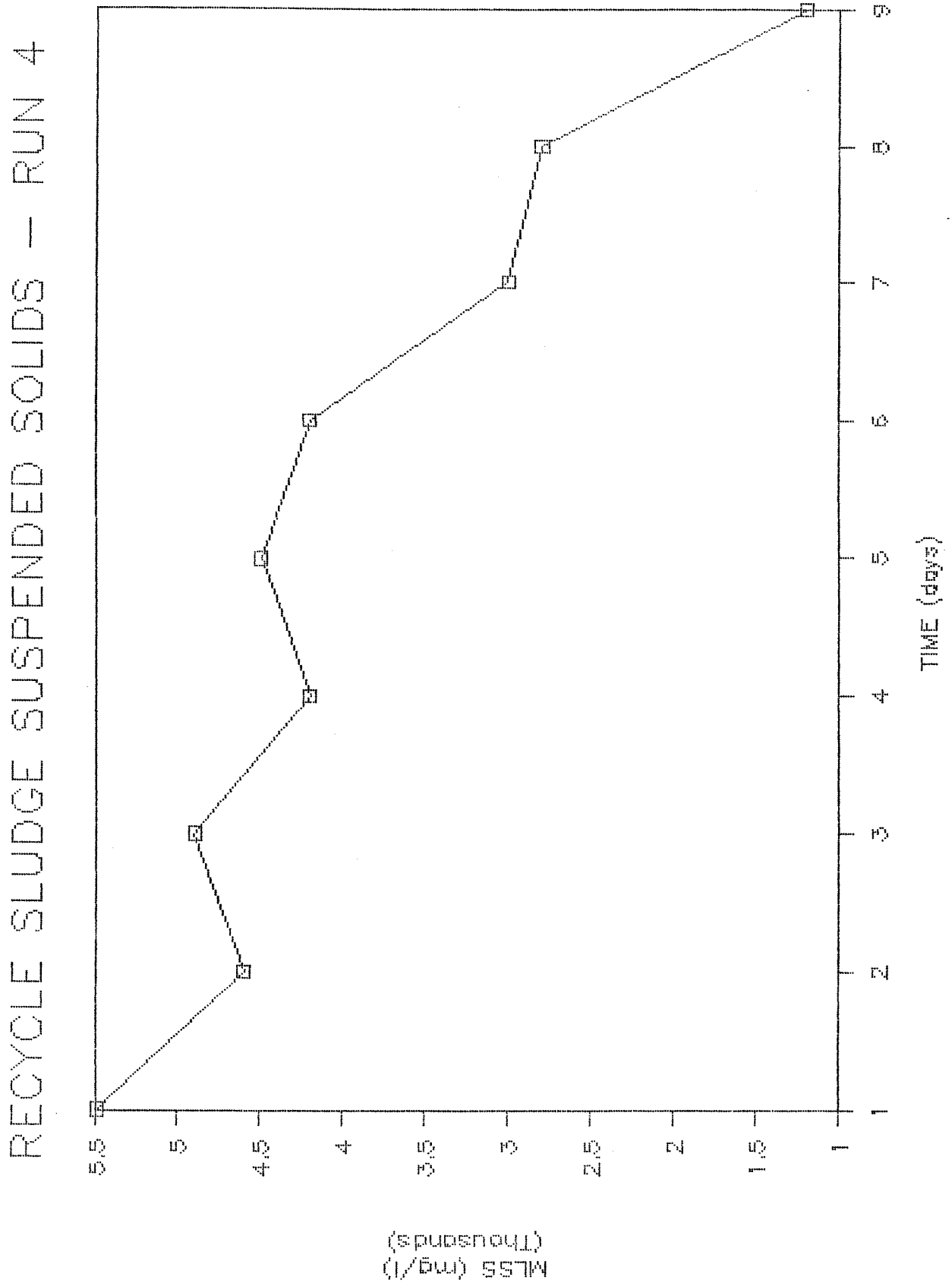

RECYCLE SLUDGE SUSPENDED SOLIDS — RUN 4
MLSS (mg/l)
(Thousands)
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
1
2
3
4
5
6
7
8
9
TIME (days)

FIGURE 19

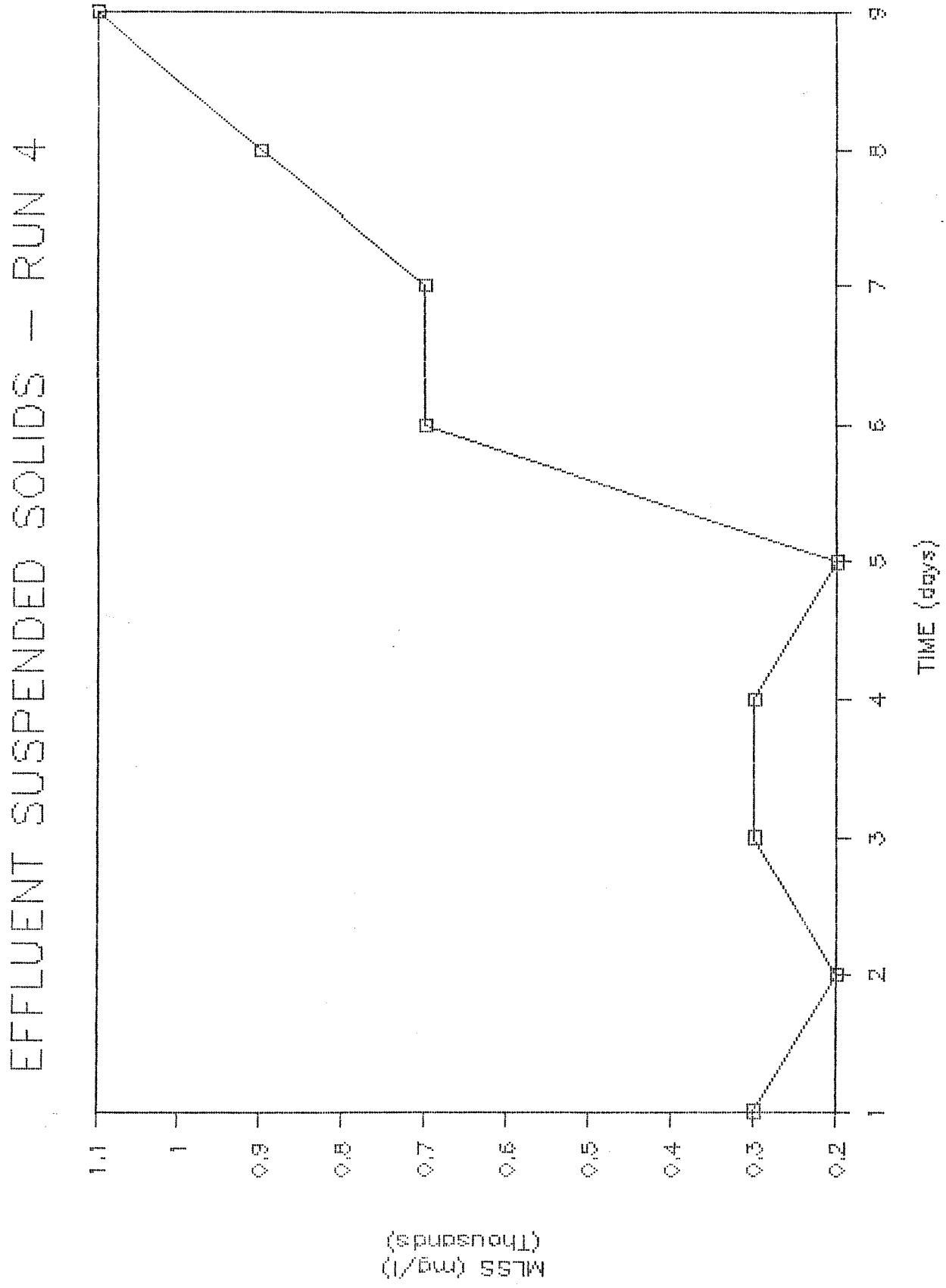

EFFLUENT SUSPENDED SOLIDS - RUN 4
MLSS (mg/l)
(Thousands)
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
1
2
3
4
5
6
7
8
9
TIME (days)

FIGURE 20

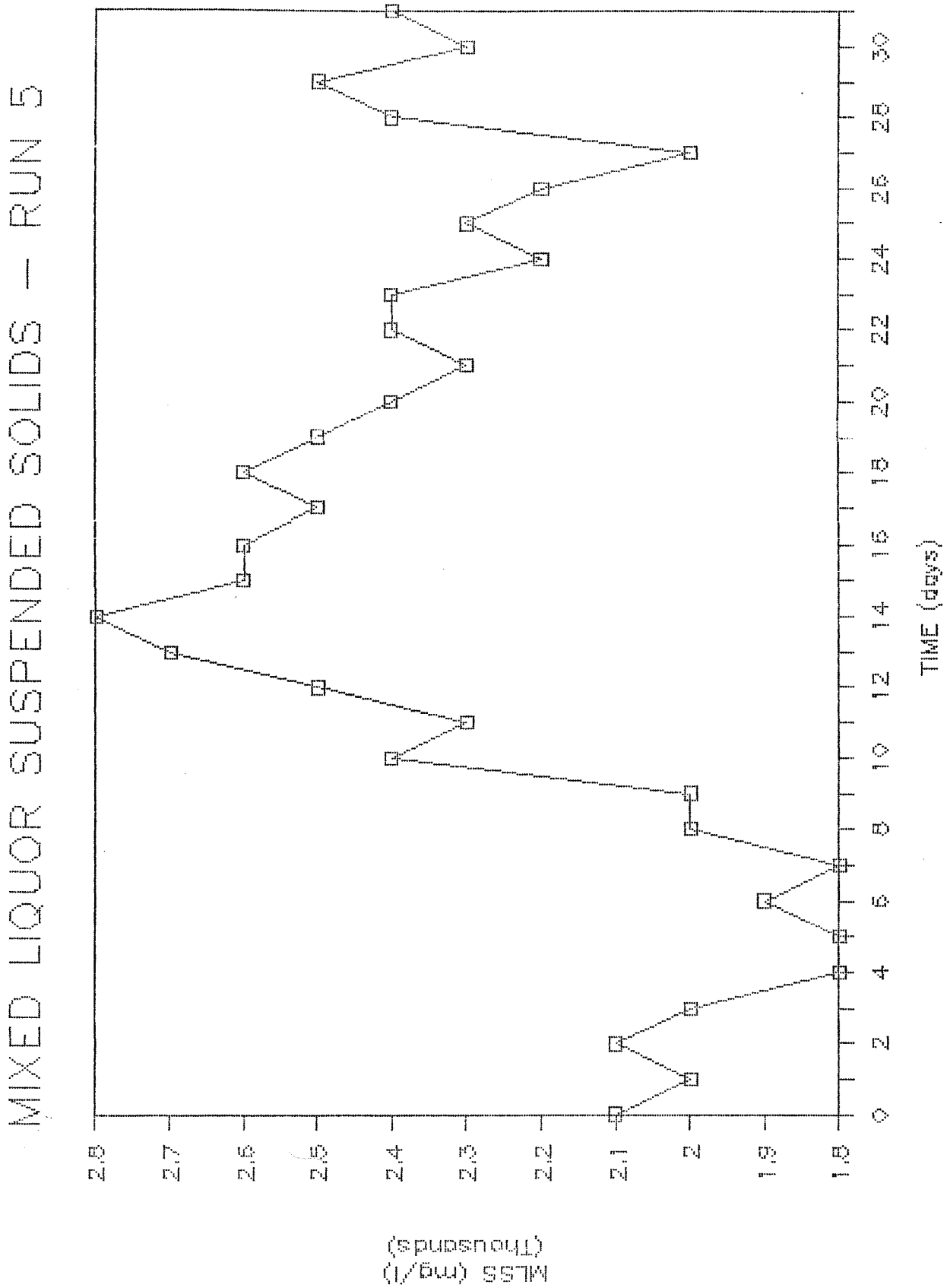

MIXED LIQUOR SUSPENDED SOLIDS - RUN 5
MLSS (mg/l)
(Thousands)
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
1.9
1.8
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (days)

FIGURE 21

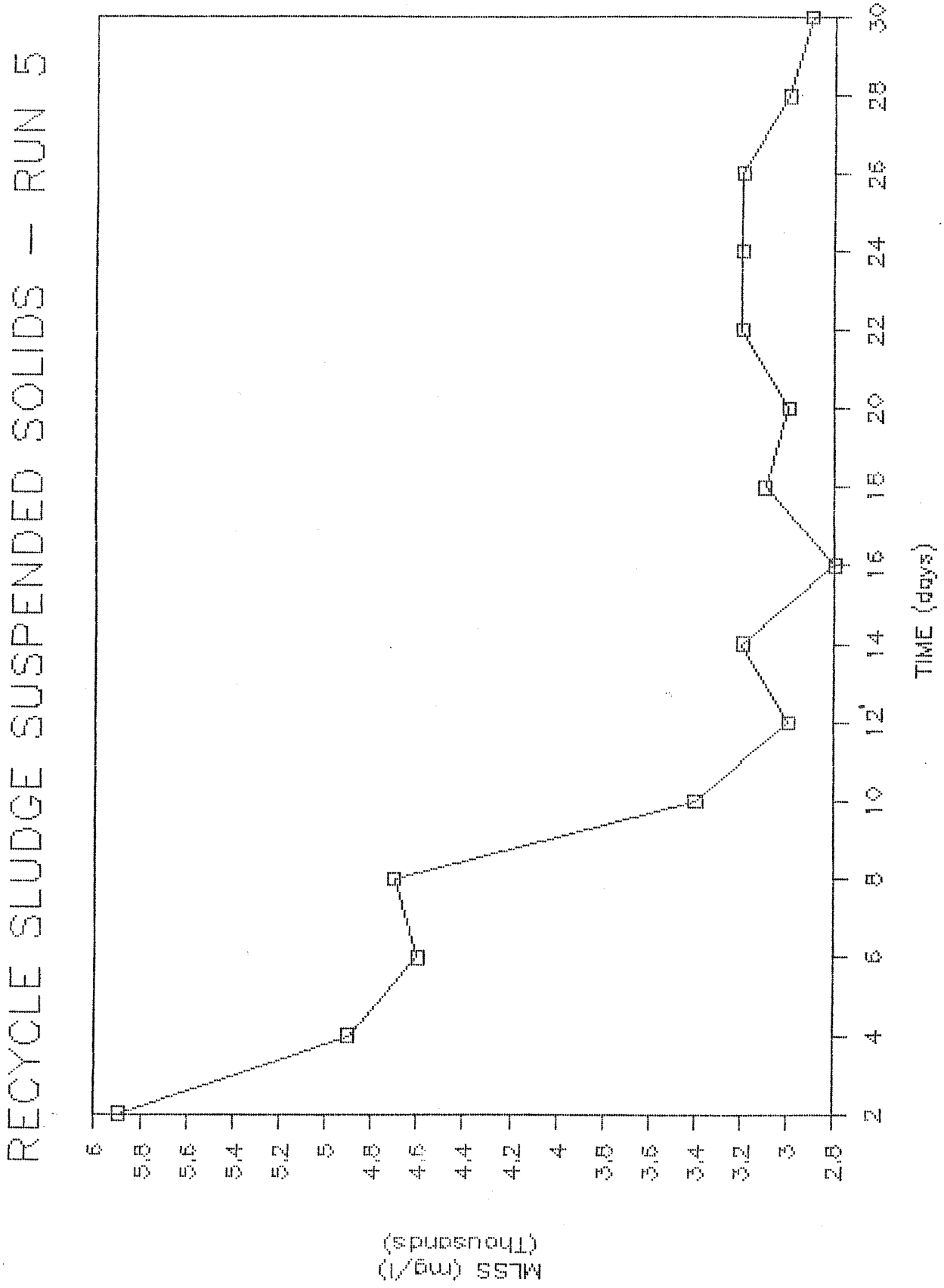

RECYCLE SLUDGE SUSPENDED SOLIDS - RUN 5
MLSS (mg/l)
(Thousands)
6
5.8
5.6
5.4
5.2
5
4.8
4.6
4.4
4.2
4
3.8
3.6
3.4
3.2
3
2.8
2
4
6
8
10
12
14
16
18
20
22
24
25
28
30
TIME (days)

FIGURE 22

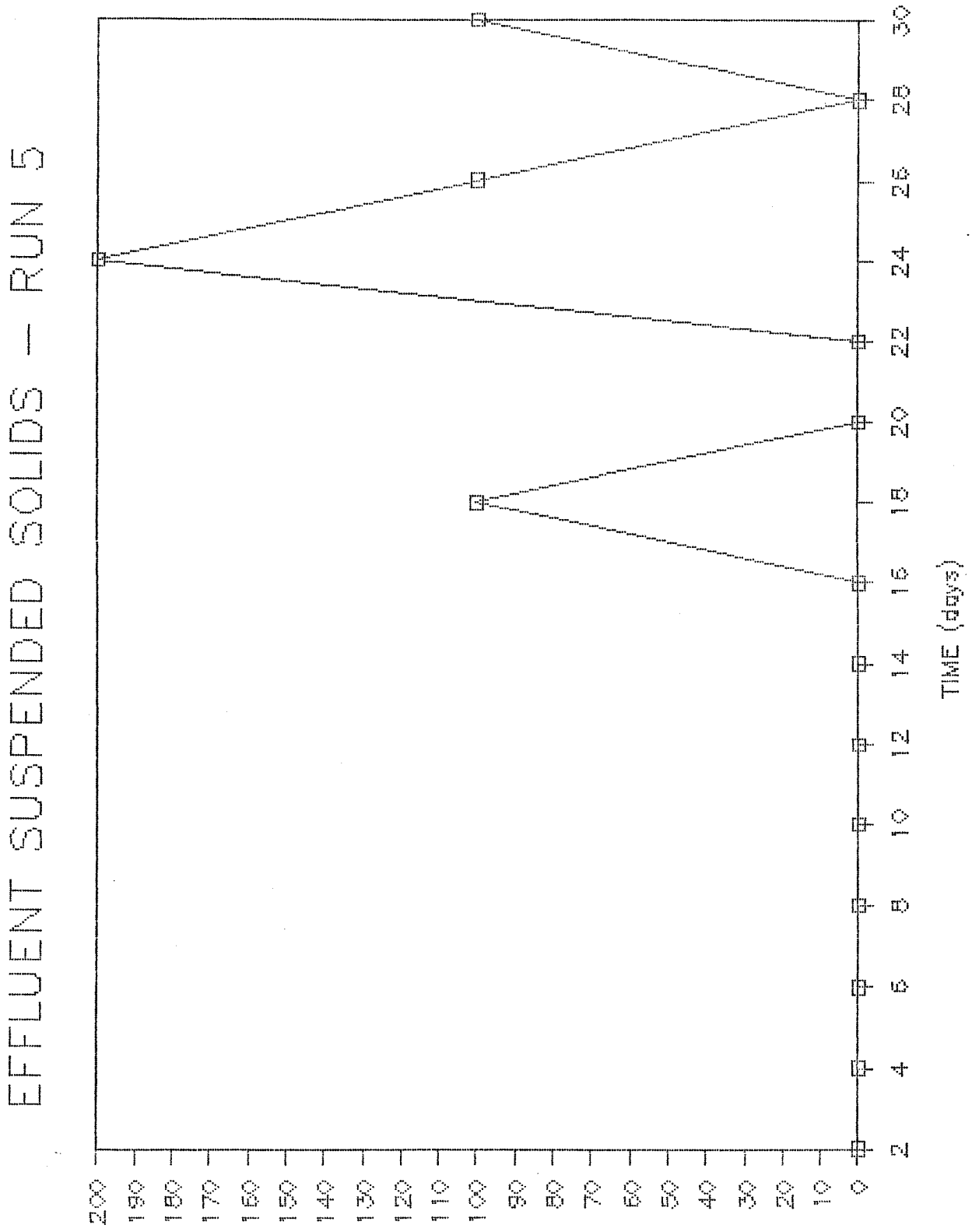
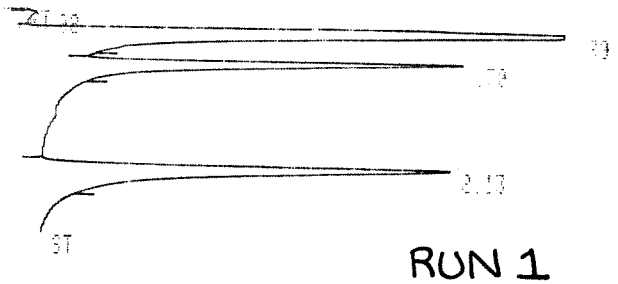

EFFLUENT SUSPENDED SOLIDS — RUN 5
MLSS (mg/l)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (days)

FIGURE 23

CHROMATOGRAM SHOWING PHENOL AND THYMOL PEAKS



RUN 1
DAY 2

RUN # 2588

ISTD

RT	AREA	TYPE	CAL #	AMOUNT
0.20	279820	BB	1	30.711
2.13	427730	PB	2%	45.450

TOTAL AREA= 707540
ISTD AMT= 4.5450E+01
MUL FACTOR= 1.0000E+00

FIGURE 24

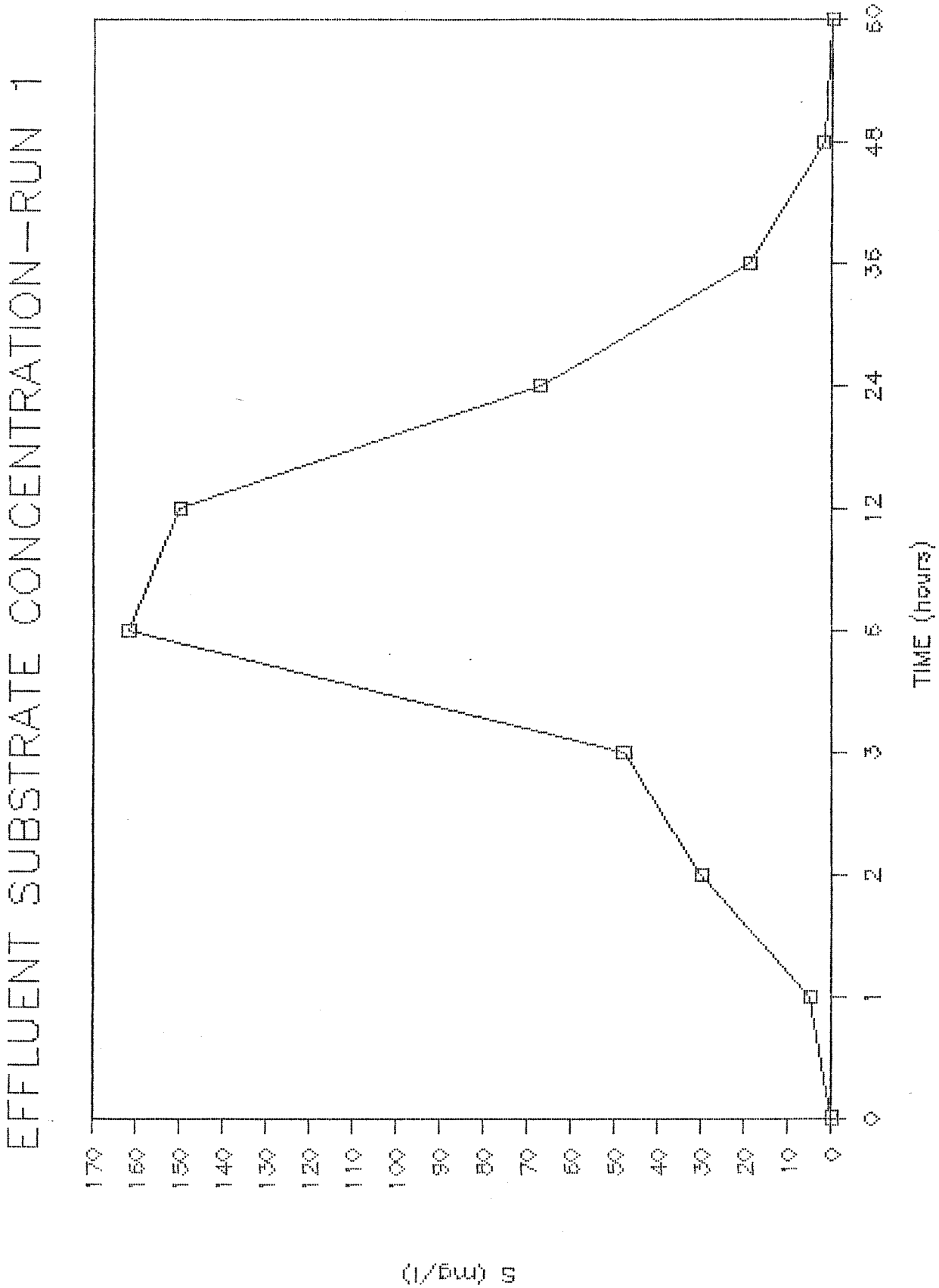

EFFLUENT SUBSTRATE CONCENTRATION—RUN 1
S (mg/l)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
0
1
2
3
6
12
24
36
48
60
TIME (hours)

FIGURE 25

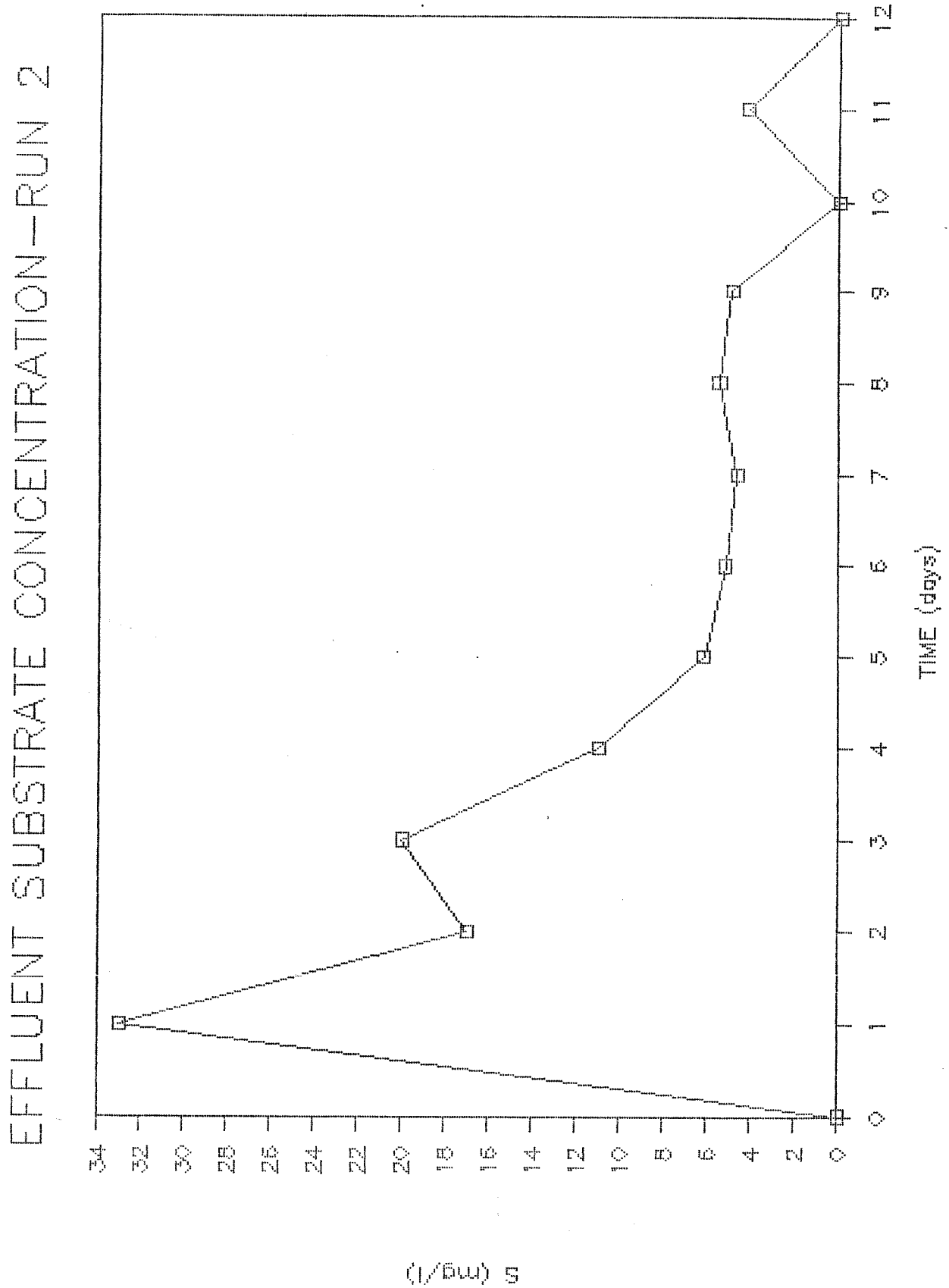

EFFLUENT SUBSTRATE CONCENTRATION—RUN 2
S (mg/l)
TIME (days)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
0
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 26

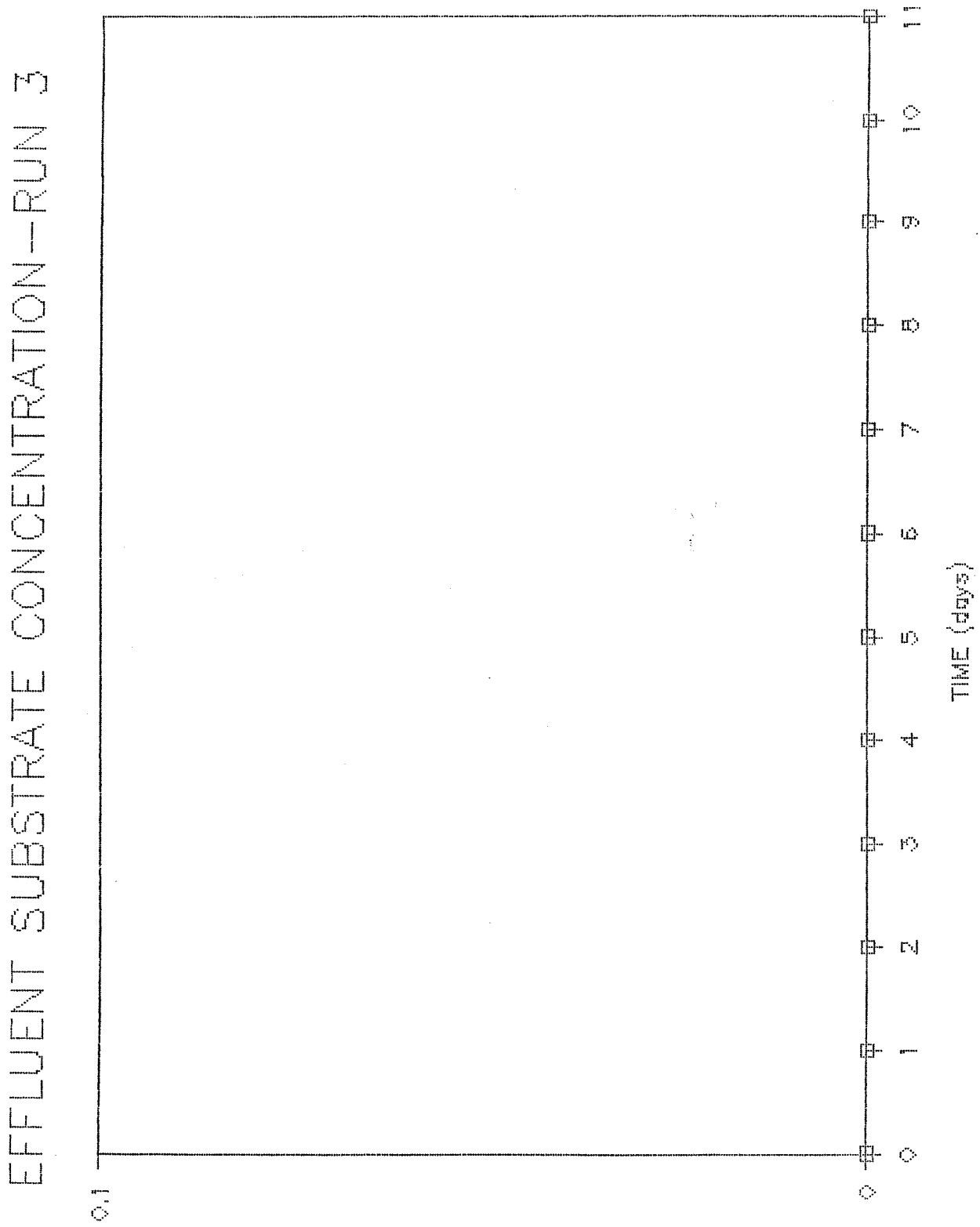

EFFLUENT SUBSTRATE CONCENTRATION—RUN 3
S (mg/l)
0.1
0
1
2
3
4
5
6
7
8
9
10
11
TIME (days)

FIGURE 27

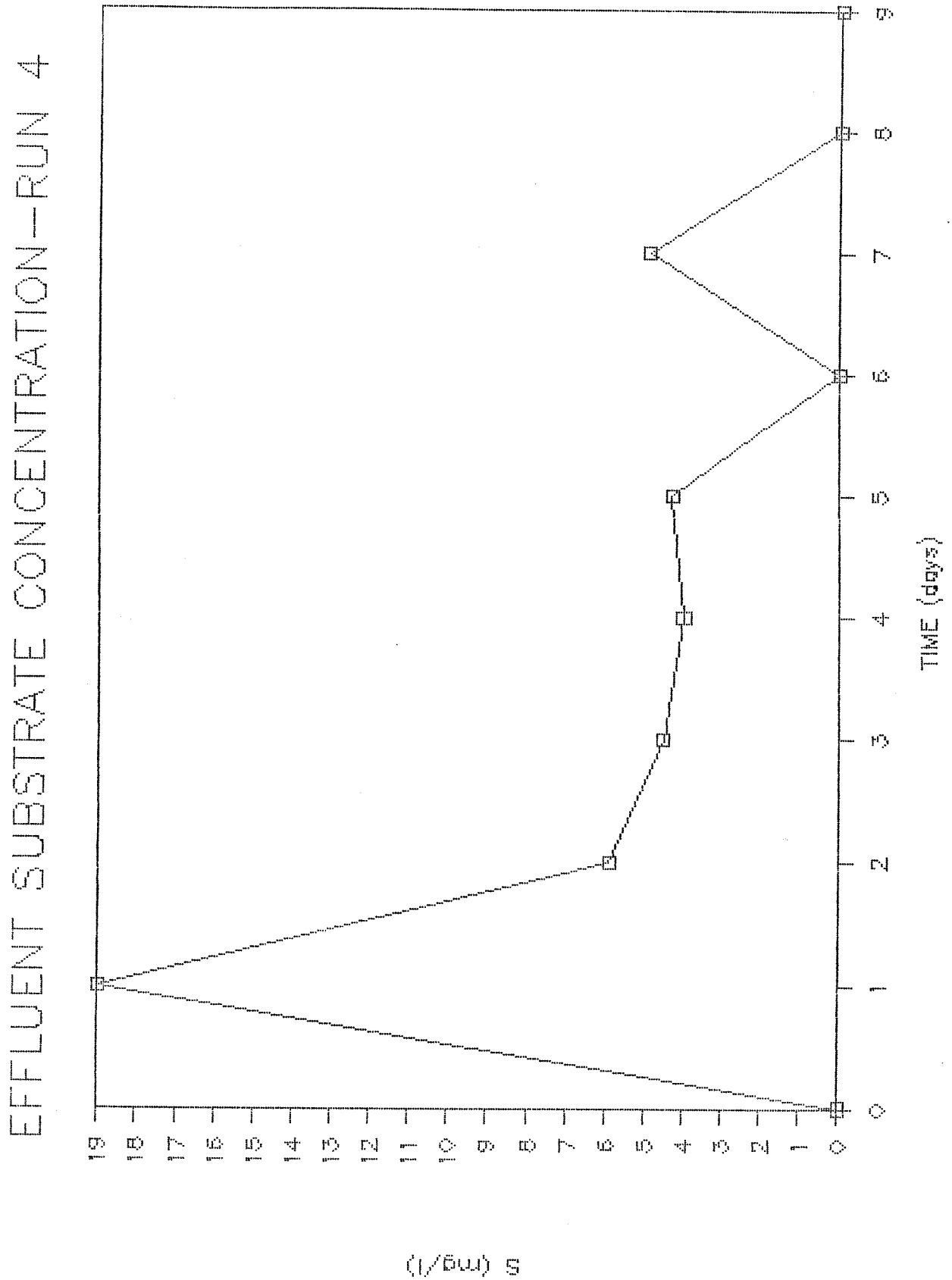

EFFLUENT SUBSTRATE CONCENTRATION—RUN 4
S (mg/l)
0
1
2
3
4
5
6
7
8
TIME (days)

FIGURE 28

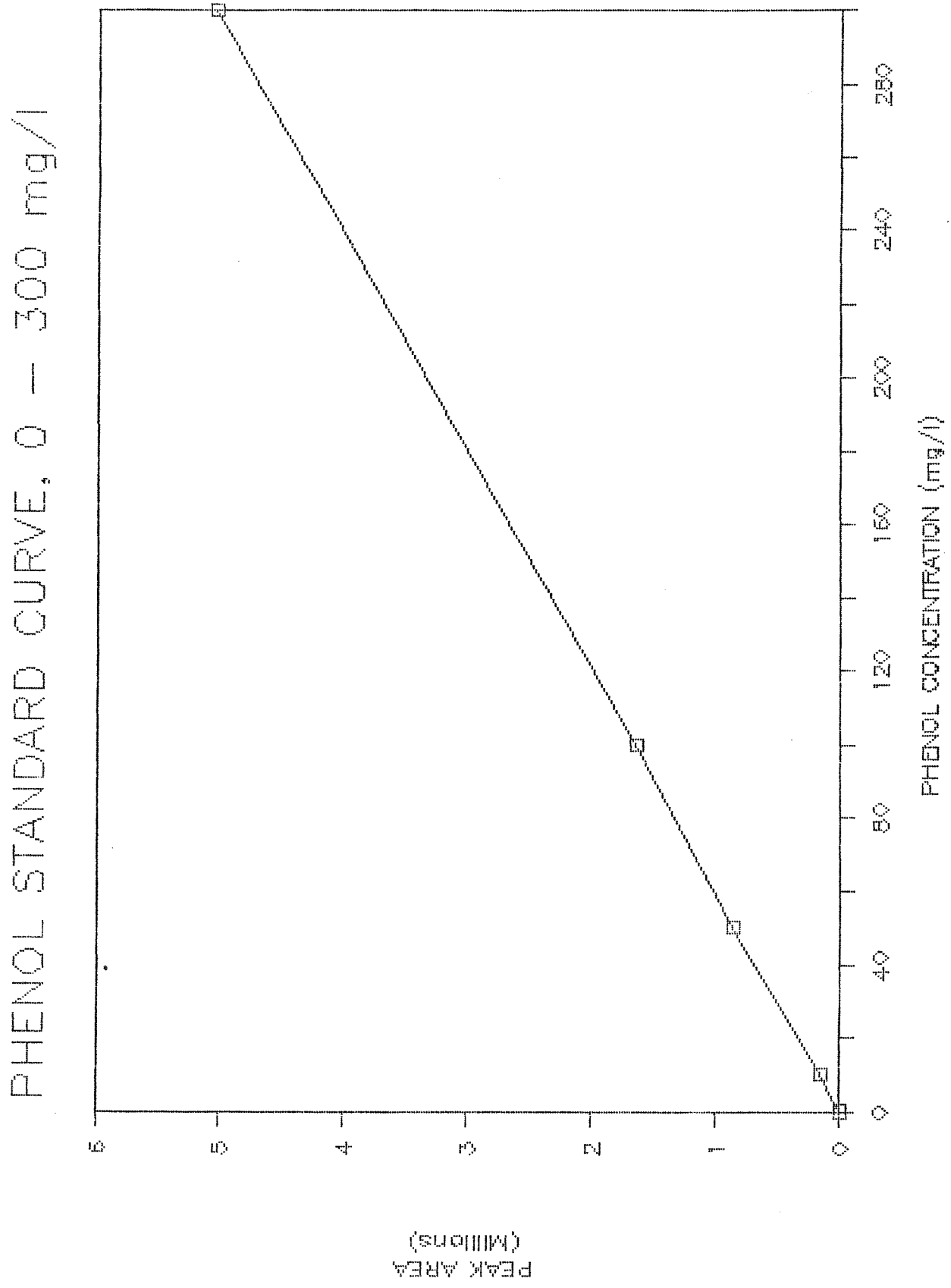

PHENOL STANDARD CURVE, 0 — 300 mg/l
PEAK AREA
(Millions)
0
1
2
3
4
5
6
0
40
80
120
160
200
240
280
PHENOL CONCENTRATION (mg/l)

FIGURE 29

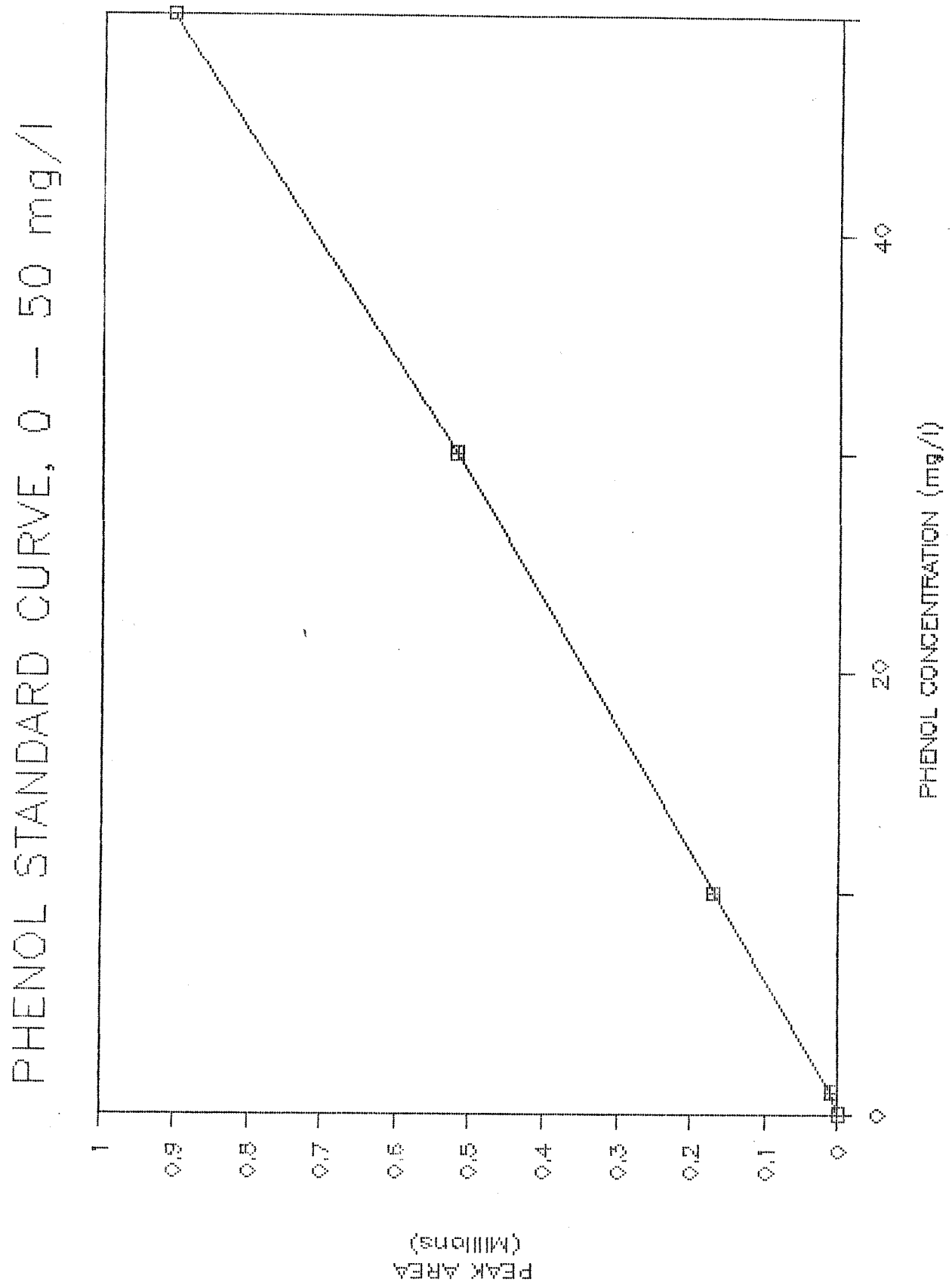

PHENOL STANDARD CURVE, 0 — 50 mg/l
PEAK AREA (Millions)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
PHENOL CONCENTRATION (mg/l)

FIGURE 30

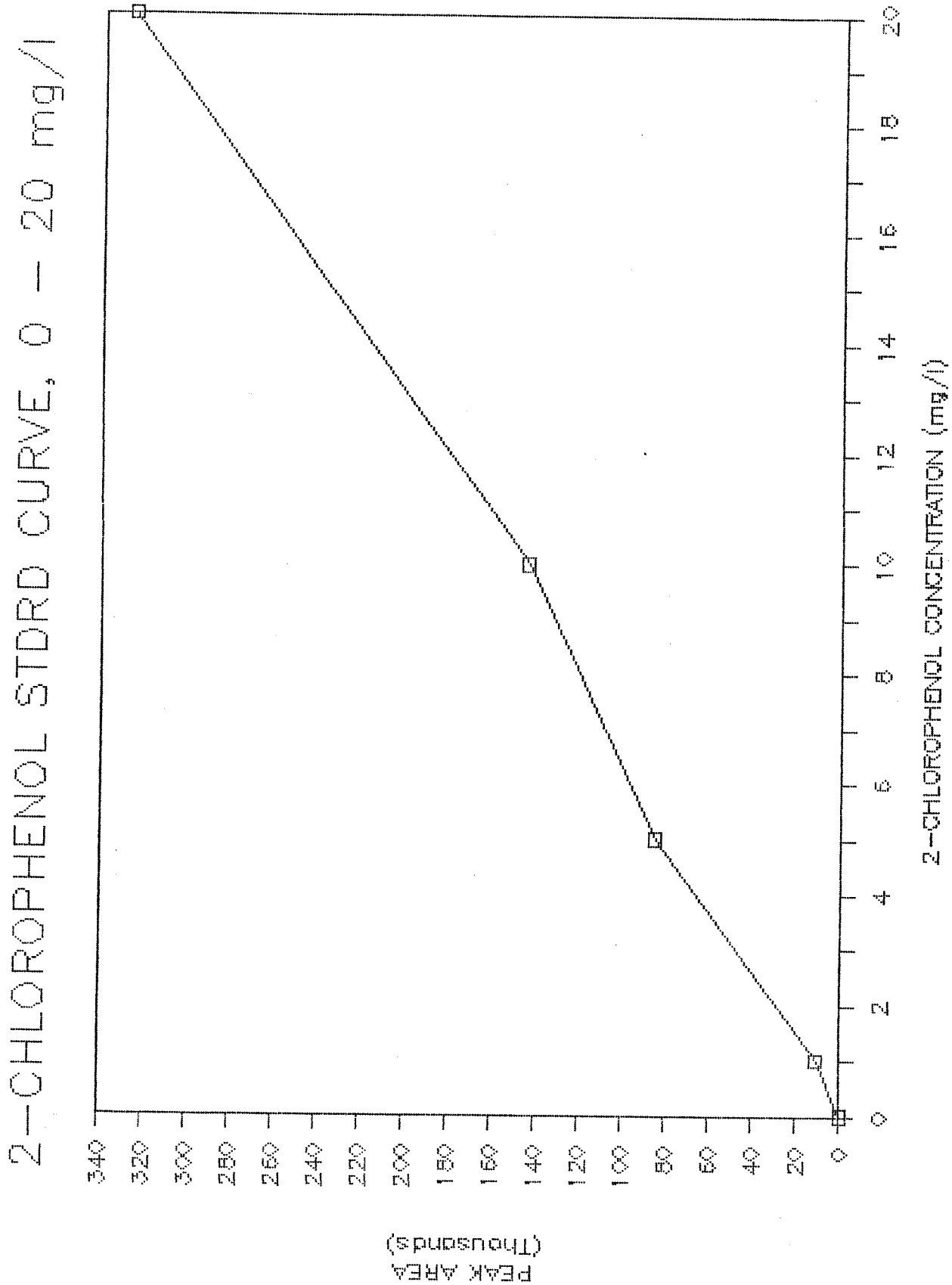

2-CHLOROPHENOL STDRD CURVE, 0 - 20 mg/l
PEAK AREA (Thousands)
2-CHLOROPHENOL CONCENTRATION (mg/l)
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
0
2
4
6
8
10
12
14
16
18
20

FIGURE 31

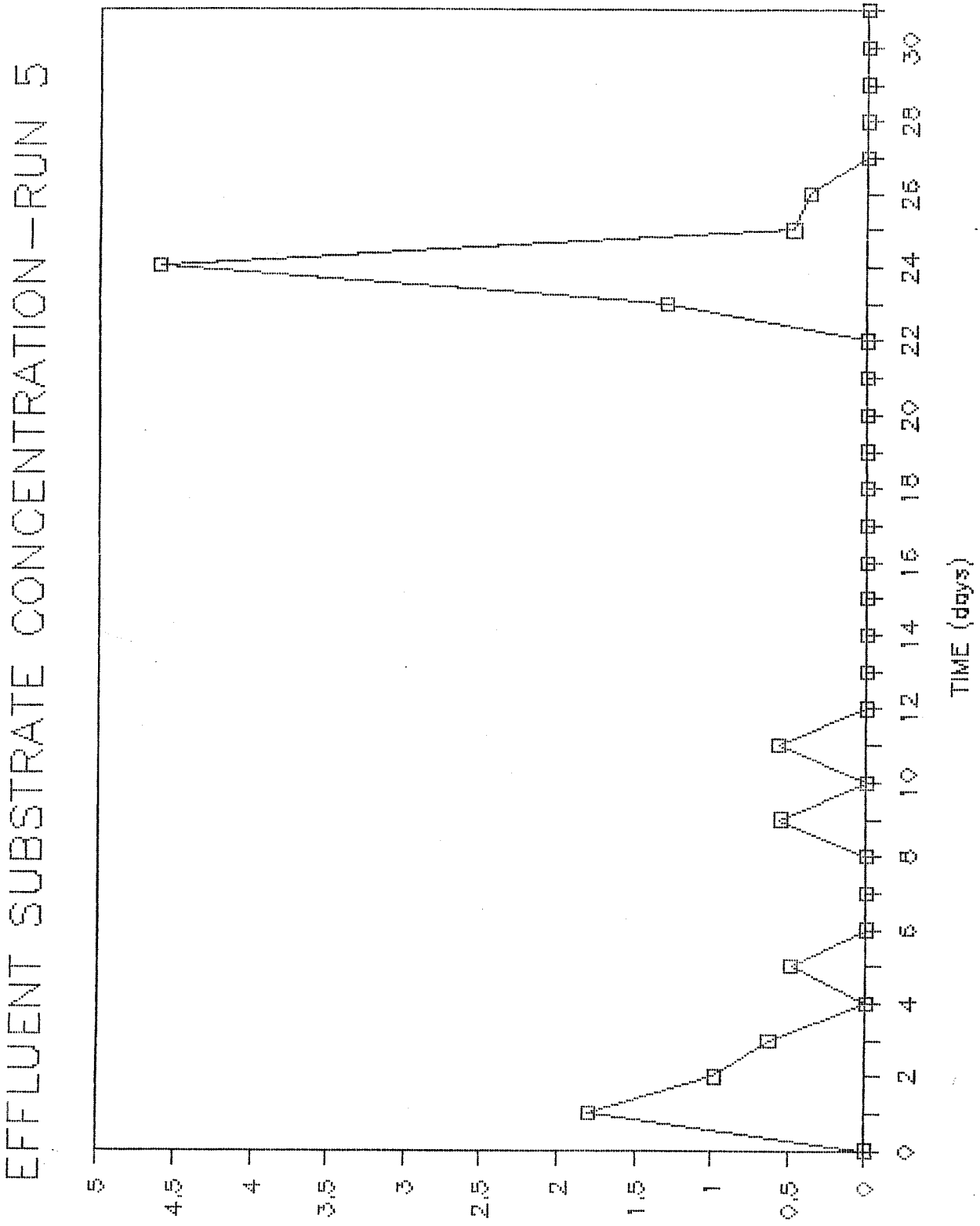
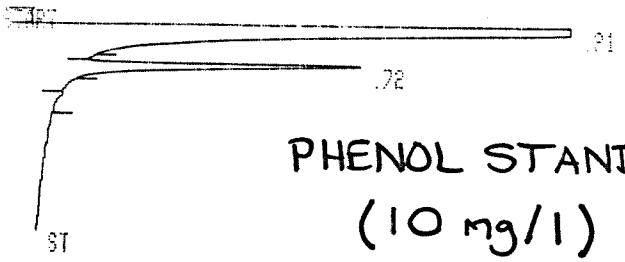

EFFLUENT SUBSTRATE CONCENTRATION—RUN 5
S (mg/l)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (days)

FIGURE 32

CHROMATOGRAMS SHOWING PHENOL STANDARD AND SAMPLE



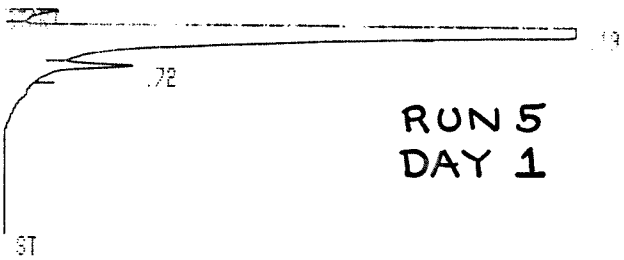
PHENOL STANDARD
(10 mg/l)

RUN # 1063

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.21	849090	PB	0.147	84.502
0.72	155720	BB	0.078	15.498

TOTAL AREA= 1004800
MUL FACTOR= 1.0000E+00



RUN 5
DAY 1

RUN # 1076

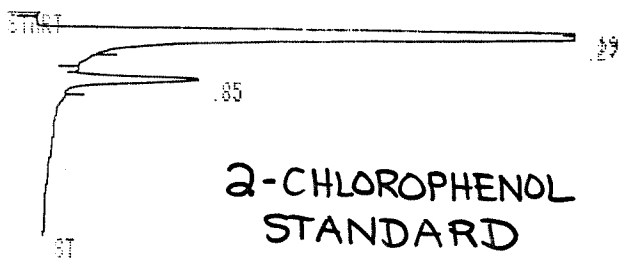
AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.19	1404500	PB	0.064	97.517
0.72	35768	BB	0.069	2.484

TOTAL AREA= 1440300
MUL FACTOR= 1.0000E+00

FIGURE 33

CHROMATOGRAMS SHOWING 2-CHLOROPHENOL STANDARD AND SAMPLE



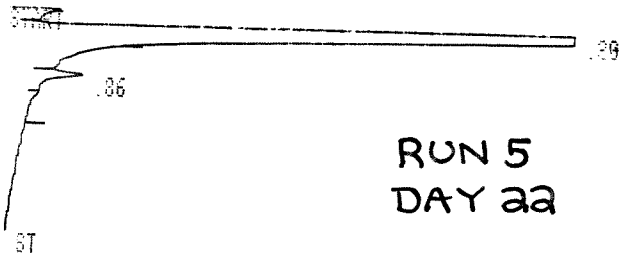
2-CHLOROPHENOL STANDARD (5 mg/l)

RUN # 1152

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.19	139220	PV	0.034	19.195
0.27	496600	VB	0.115	68.468
0.85	89485	VB	0.098	12.337

TOTAL AREA= 725310
MUL FACTOR= 1.0000E+00



RUN 5
DAY 22

RUN # 1167

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.20	1218100	PB	0.083	98.671
0.86	16407	BP	0.070	1.329

TOTAL AREA= 1234500
MUL FACTOR= 1.0000E+00