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THE BIOCHEMICAL FUEL CELL: CONVERSION OF WASTE TO ENERGY

by

CHARLES AUGUST MIELKE JR.

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 Chemical Engineering
1986

APPROVAL SHEET

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ABSTRACT

Title of Thesis: The Biochemical Fuel Cell: Conversion of Waste to Energy

Charles August Mielke Jr, Master of Science in Chemical Engineering, 1986

Thesis directed by: Professor Dr. T. Greenstein

A batch biochemical fuel cell was constructed and studies on the production of bioelectric energy using an alga Scenesdesmus quadricauda, and a bacterium Desulfovibrio desulfuricans, were conducted. Results were compared for a steady state cell using various concentrations of media components and showed that a 1000-fold increase in the concentration of media components produced a net potential output of 0.03mv/ml compared to an initial output of 0.02mv/ml. Further studies showed the biochemical net potential output using activated sludge from a waste treatment facility was 0.01mv/ml compared to 0.02mv/ml for the cell using the initial media concentration.

The system exhibited steady state conditions within 8 to 10 hours of inoculation. Output remained fairly constant for a period of 14 hours and then decreased. The specified methods were carefully duplicated for each experiment including the sterilization of equipment, accurate weighing of raw materials, and monitoring of temperature. Thus the output potential recorded was

based on the fuel cell volume, biological activity of the micro-organisms and the media component concentrations.

The activated sludge fuel cell is a viable one even with a reduced output of 0.01mv/ml.

FOREWORD

Fuel cells have only recently begun to be applied in research and industry as potential suppliers of energy. While the initial potential was started in early NASA days, in the form of biologically decomposing human waste, the prospect of converting waste to energy is slowly developing. With the shortage of energy in the world becoming greater, the need for developing wastes of all kind into energy, via the technological application of biochemical engineering is ever great.

The need for biological expertise in understanding and learning the special techniques for handling and propagating the microorganisms was fulfilled by Dr. H. Rubin. I wish to thank Dr. Rubin for his guidance and support in the conduction and completion of this thesis. Secondly I would like to thank Dr. T. Greenstein for his support, guidance, patience and idea which started the basis for my thesis. Thirdly I would like to thank Dr. R. Tompkins for supplying the platinum wire that was fabricated into an electrode. Last but not least I would like to thank my family, Lynn; my wife, who was behind me every step of the way, and children, Ellen and Mark, who will one day realize what it takes to prepare a thesis when they themselves prepare one.

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INTRODUCTION

The development of fuel cells has long been a curiosity of the scientific community, particularly in terms of commercial application. Recently, however, an interest in the biochemical fuel cell and its ability to generate electrical energy has gained momentum.

A biochemical fuel cell is a fuel cell in which electrode reactions are promoted or catalyzed by biological processes. A clear understanding of the metabolic processes in a biochemical fuel cell is only just beginning to be achieved. Space exploration has stimulated fuel cell work in a concerted effort to utilize waste products as reactants for producing electricity. The properties which characterize biochemical fuel cells may be summarized as follows:

1) Mild conditions of electrolyte concentration and temperature
2) A variety of complex and unusual reactants (fuels) can be effectively utilized such as natural products, vegetable or animal matter
3) Regeneration may be continuous, thereby the cell would have a long life

While several biochemical fuel cells have been developed to produce electrical energy, few have been able to sustain it and with little power. Thus the future objectives of fuel cell technology is to provide methods for increasing the electrical output and longevity of a

biochemical fuel cell. The possibilities of numerous micro-organisms, fuel cell volume, nutrient concentrations, cell concentration and fuel cell design are endless. This research work is primarily concerned in determining the optimum nutrient concentration for a biochemical fuel cell. The secondary focus of this work is in the application of a readily available waste, activated sludge, in producing electricity in a biochemical fuel cell. The first recorded bioelectrical studies were made by Galvani (5) in 1786, where he discovered electricity in frogs' muscles. More recently, the first investigations of biochemical fuel cells were made by M.C.Potter(5) in 1912 and by B.Cohen(5) in 1931 who measured the potential differences between various cultures and sterile media. Potter, in particular, observed that the electric current and potential differences were associated with the oxidation of glucose in the presence of bacteria. Similiarly, Cohen, found that disintegration of organic compounds by micro-organisms yielded electrical energy. This interest in the decomposition of organic wastes, as a biochemical cell, was stimulated by U.S. Government research. In the early 1960's, the possibility of providing human waste disposal in space stirred great interest in biochemical fuel cell research. Later investigations by commercial companies into the generation of electricity from organic wastes was documented by numerous patents given to major oil

companies.

Various biochemical fuel cells were developed from these patents. These include 1) different combinations of micro-organisms and 2) fuel cell structure and configuration. The early work involving the living organism or their enzymes in the sea to produce a sea battery was done by G.H.Rohrback(9). Six various fuel cell and electrode configurations were evaluated by Rohrback in his work. In addition the development of a bacteria coated electrode to enhance electrical production was made. At Mobil Oil, Davis et al(3) experimented with micro-organism combinations to produce an electromotive force(EMF) range of 140-380mv, with a current of 0.25 to 4.5 milliamperes of current. Sutton et al(12) continued with much the same work for the U.S. Government. His biochemical-chemical fuel cell also employed various micro-organisms in one half compartment with the other electrolyte containing no organisms. The electrode output of this cell design produced a range of 0.02 to 0.09 volts with a current of 10 to 700 microamperes respectively. Nelson Alexander(1) continued with the same work employing a fuel cell with both compartments containing micro-organisms. The purpose of this study was to continue Alexander's work by determining the optimum nutrient concentration for a given micro-organism combination. In addition the application of an abundant waste as a fuel to the same cell configuraion,

was attempted.

The concept of biological systems as fuel cells can be regarded as an electrochemical energy converter.[1] One half of the fuel cell oxidizes food, the other reduces oxygen, ions pass through the solution, and there is electron transport in the cell. Given this concept of understanding biological reactions in terms of electrodic reactions, we can divide biochemical fuel cells into two main types. The indirect biochemical fuel cell (IBFC) where the primary fuel fed to the organism is converted into a waste product which can be removed and utilized in a separate fuel cell. Examples of this are the production of hydrogen from carbohydrate by the organism Clostridium cellobioparus; hydrogen from formic acid by Escherichia coli, ammonia from urea by, among others, Bacillus pasteurii; and ethanol from carbohydrate by Saccharomyces. In addition IBFC processes can also be adopted to producing oxidant; examples are the production of oxygen from carbon dioxide by algae and plant-life in general (the photosynthesis process) and the production of oxygen (and nitrogen) from nitrate by Micrococcus denitrificans. As a general rule it can be assumed that fuel production is a consequence of exergonic processes (i.e. processes taking place with energy liberation) while production of oxidant takes place as a consequence of endergonic processes (i.e. processes taking place with energy absorption). In the first case of

IBFC, the organism utilitzes part of the energy content of the primary fuel; in the second case the organism must be supplied with a secondary energy source, e.g. light. The direct biochemical fuel cell (DBFC) utilizes the same basic processes as the indirect fuel cell but in this case the organism can function two ways. It may be used to provide a continuous supply of the enzymes required by the biochemical/electrochemical process, in which case, since the organism is allowed to derive no benefit from the process, it perishes. Therefore a portion of the population must be allowed to grow in the normal way (consuming some of the fuel in the process) so as to ensure the continued growth of the organism. Alternatively, the organism may be grown on or in the close neighborhood of the electrode and the waste product of its metabolism (ammonia, ethanol, hydrogen, etc.) utilized directly for the production of electrical energy.

With the concept of the flow of electrons by the chemical conversion of organic materials proven, more complex investigations such as differences in electrical output with no changes in bacterium and various physical apparatus configurations were executed. Most of this developmental work however combined both apparati and bacterium changes simultaneously, rather than evaluating the effects of keeping all but one variable fixed.

The concept of ionic transport serves as a basis for

the physical apparatus of most biochemical fuel cells. Two compartments or chambers, each containing its respective electrolyte, is separated by a barrier. As previously mentioned, various configurations by Sutton et al(12), Taylor etal(13), Davis et al(3), were attempts to determine the effects of fuel cell configuration. The barrier is a salt bridge, which provides a means to allow separate reactions in each of the two compartments, while providing a path for electron transfer.

The fuel cell described herein is of the same design concept. In one compartment of the fuel cell an oxidation reaction takes place while a reduction reaction occurs in the other half. These separate reactions result in a flow of electrons through the barrier, which is measured by a meter. The reactions taking place in each half cell can be described as follows: a generation of hydrogen ions at the anode is formed by the oxidation of the sulfate compounds in the medium. At the cathode the oxygen is reduced under the catalytic influence of the micro-organism. Thus the hydrogen ion formed in the anode or negative electrode migrates to the electrolytic solution of the other, forming the internal circuit. Alternately, electrons flow from the anode half-cell to the cathode half-cell forming the external circuit. The electrical output is the resultant completed circuit read at the meter.

For the cell studied, the Scenedesmus alga generates oxygen

due to the photosynthesic metabolic process of the alga in the presence of fluorescent light. The generated oxygen acts as an electron sink in the presence of the platinum anode and the electrons are absorbed at the positive electrode. Due to this absorbtion, hydroxyl ions are produced, reflecting a saturated oxygen electrode in the presence of an electron deficient electrolyte.

The ion cathode immersed in the anaerobic electrolyte tends to form spontaneous metallic ions and free electrons. The oxidation of the ion is accompanied by the generation of hydrogen, the normal reaction being:

$$H_2O + e \longrightarrow 1/2H_2 + OH^-$$

The biological organism, Desulfovibrio desulfuricans, inoculated in the electrolyte, acts as a depolarizer by consuming the generated hydrogen as follows:

$$SO_4^{2-} + 4H_2 \xrightarrow[\text{desulfuricans}]{\text{Desulfovibrio}} HS^- + OH^- + 3H_2O$$

Thus the generated hydrogen causes the potential of the negative electrode to approach that of a saturated hydrogen electrode. The overall reaction is as follows:

$$5H_2O + SO_4^{2-} + 8e \xrightarrow[\text{desulfuricans}]{\text{Desulfovibrio}} HS^- + 9OH^-$$

When the positive and negative terminals are connected to the resistance of the measuring circuit, the resulting flow of electrons initiates polarization in the form of excessive accumulation of hydrogen gas on the cathode,

partially insulating it, and depletion of the accumulated oxygen on the anode. The rate of microbial depolarization, represented by the consumption of hydrogen at the cathode or generation at the anode, determines the output of the cell.

As previously discussed, the direct transfer of electrons from micro-organims to electrodes has been demonstrated in a microbial fuel cell. However, this process is somewhat inefficient. Currently, both the rate of electron transfer (the current available) and the proportion of electrons transferred (the coulombic yield) for biochemical fuel cells are at low levels. Therefore commercial applications are limited. Thus, in terms of producing greater electrical output, an investigation into increasing the efficiency rate and proportion of electrons for biochemical fuel cells must be made. Present work involves the use of mediators, which can be both rapidly reduced by the micro-organism and rapidly re-oxidized at the electrode and may provide a mechanism for improving electron-transfer efficiency.

Improvements in electron transfer efficiency is one attempt at improving the electrical output of biochemical fuel cells. Continuing investigations will enable the biochemical fuel cell to used in the following applications:

1) Power sources in remote locations such as underdeveloped countries
2) Conversion of human wastes to electricity which would lead to self sufficient waste treatment facilities
3) Space power from human wastes on space stations

MATERIALS AND METHOD

FUEL CELL CONSTRUCTION

The design of the biological fuel cell is relatively simplistic. A "U" shaped casing was made from glass tubing. Two 254mm long by 76.2mm diameter pieces of Pyrex glass were connected on the bottom by a 76.2mm long by 25.4mm diameter piece of glass. Each connecting joint was fused to produce a smooth non-obstructing 25.4mm diameter passage. An analogy would be two vertical mine shafts connected by one horizontal shaft allowing full passage. The bottom of both 76.2mm diameter cylinders were fused shut to create a water tight housing. All glass work was performed with a wide flame Bunsen burner using natural gas as a fuel.

To allow for biological cell growth, yet to isolate the open top cylindrical compartments from outside contaminants, closures were developed. The aerobic compartment has a loose fitting cover with sides turned downward, similiar to the lid of a petri dish, to allow for gas exchange. The other cylinder has cut ground flat surface and a matching flat ground lid that creates a tight seal for anaerobic growth.

An additional feature of each cover was the installation of a teflon compression fitting in the center of each lid. This fitting allowed the insertion of tubing

or wires through its center and when a retaining nut was tightened the tubing and/or wire was locked in place. The purpose of the compression fitting was to hold the electrodes in the center of each compartment and, in the case of the anaerobic compartment, to provide a tight seal around the electrode when the small inner teflon ferrule within the fitting was compressed in place around the electrode itself.

Two of these cell compartments were made in the glass craftsman's shop at Merck Sharpe & Dohme research laboratories. A detailed rendering of the cell is shown in figure I.

The electrodes used in this fuel cell were constructed of platinum and iron. The cathode, or iron electrode, was constructed by taking a 254mm long, 0.508mm diamter piece of iron wire and wrapping it around a wooden dowel to form a spiral. One inch of wire was left straight to allow insertion into the cover compression fitting. The anode was constructed of platinum wire 254mm long, 0.508mm diameter and again formed into a spiral shape with a one inch straight terminal section. The electrodes were of similar length and diameter to provide an equal surface area for ion exchange, thus, eliminating the possibility of an unequal potential.

From the tip of each electrode above the compartment

cover, a copper wire, 16 gauge uninsulated, was crimped to the electrode. These two conductors, one from the anode and one from the cathode were connected to a electrometer to complete the external circuit of the cell. From the electrometer a strip chart recorder was connected to record the fuel cell's continous output.

GROWTH AND MAINTENANCE OF MICRORGANISMS

The microorganisms used in the fuel cell were the same as those used by Nelson Alexander(1). Scenedesmus quadricauda, ATCC 11460, a blue-green bacteria, was the aerobic microorganism. Desulfovibrio desulfuricans was the anaerobe. The two organisms were obtained from Merck, Sharp & Dohme's culture bank.

Initially the species were inoculated into an aqueous electrolyte media. Table I lists the composition of this medium.

The algae were grown in the electrolyte medium in erlenmeyer flasks at 37 C. A 75 watt fluorescent lamp was left on continously at a distance of 24 inches from the flasks. To maintain the algae culture, solid medium was prepared by adding 1% agar to the electrolyte medium, inoculating with Scenedesmus quadricauda and then incubating at 37 C in the presence of the fluorescent light. The cultures were maintained regularly by weekly transfer onto fresh media.

For Desulfovibrio desulfuricans, Alexanders Aqueous Electrolyte (AAE)(1) was used. Its composition is shown in Table II. To promote the growth of this anaerobic micro-organism an anaerobic gas pack jar, (model GasPak 150,BBL) or candle jar was used to produce a carbon dioxide rich atmosphere. Seven attempts were made to initiate growth using AAE, with no success, thus Starkey's Desulfovibrio medium listed in Table III, was used. Desulfovibrio grew well in this medium at 37 C. To maintain the culture, sufficient agar was added to make a 1% medium, allowed to solidify in petri dishes, inoculated, and incubated in a candle jar, at 37 C. For each experiment, fresh media was prepared and added to the appropriate compartment of the fuel cell.

FUEL CELL SETUP

For each experiment, the bioelectric cell casing and covers were sterilized at 121 C for 15 minutes. Next, a salt bridge consisting of a solution of 5% sodium chloride and 10% agar in distilled water was sterilized and then added to the cell casing allowing it to completely fill the interconnecting passage between the two cell compartments. The salt bridge was allowed to cool and solidify at room temperature. The two liquid media were then poured into their respectitive cell compartments and allowed to cool to prevent dissolving the formed salt bridge. After the media cooled, sterilized cotton was added to the anode, or algae compartment, to act as a growth template. The algae was

added to the anode by placing it directly upon the cotton and then covering the compartment. The cathode was outgassed with nitrogen for five minutes prior to inoculation with Desulfovibrio.

MEDIUM CONCENTRATION MODIFICATIONS

Experiments were performed changing the medium concentrations of each electrolyte and evaluating the potential. The first trials were performed with a ten fold increase in the media concentration of both half cells. First the medium was prepared and the casing was set up with its agar barrier. The electrolyte solutions were then added to the cell. The same control experiment was performed in that the output of the cell was measured before inoculating and then measured again with the half cells inoculated with microorganisms. For the algae half cell sterilized cotton was used as a support for the organism. The anaerobic half cell was outgassed with nitrogen for five minutes after inoculation at a rate of two cubic feet per minute to remove oxygen. The electrodes were then placed in position and the electrometer was turned on along with the strip chart recorder.

Two more sets of experimental trials were performed with the media concentrations increased to 100 fold and 1000 fold levels, respectively. For these trials, the cell was prepared as previously and then the electrolytes were

prepared. The cell casing was sterilized and the salt bridge was added. The electrolyte solutions were added to their respective compartments, covers put in place with the electrodes, and the measuring device and strip chart recorder turned on. After the cell was evaluated without the microorganisms, the cell was again prepared with the compartments set up to take an inoculation. For the Scenedesmus quadricauda algae sterilized cotton was used as a medium support for the organism. The Desulfovibrio desulfuricans compartment was outgassed with nitrogen at a rate of two cubic feet per minute for five minutes after inoculation to remove oxygen. The load bearing device was turned on along with the strip chart recorder and data taken.

ACTIVATED SLUDGE FUEL CELL

FUEL CELL CONSTRUCTION

The design of the activated sludge fuel cell is simplistic as in Figure II.The same "U" shaped casing made from glass tubing was used. The two 76.2mm diameter cylinders were connected by a 25.4mm diameter piece of glass. Like the previous cell construction, the glass work was performed with a wide mouth flame bunsen burner using natural gas as a fuel. The housing was made watertight by fusing all ends of tubing.

Closures for each open top compartment were developed

to allow for biological cell growth yet to isolate each compartment from outside contaminants. The aerobic closure has a loose fitting cover with sides turned downward to allow easy gas exchange. The anaerobic closure had a cut ground flat surface and a matching flat ground lid that created a tight seal. The additional installation of a teflon compression fitting into the center of the lid was made. This compression fitting allowed for the insertion of wires or tubing through its center. In this way the compression fitting held either an electrode or tubing in place yet provided an airtight seal when the fitting was torqued down.

In the activated sludge fuel cell an additional feature was added to the sludge compartment closure. To provide for the subsurface air distributor a second compression fitting was added to the closure. The subsurface air distributor was then placed at the desired height from the bottom of the compartment by tightening the compression fitting. The air distributor was constructed of 6.35mm diameter stainless steel tubing bent into a circular ring approximately 50.8mm in diameter and having an overall length of 304.8mm. For air distribution into the sludge 0.8mm diameter holes were drilled into the circular tubing at equal spacing around the circumference.Figure III illustrates a detailed rendering of the distributor along with the special closure. To the end of the distributor

tubing, extended up through the compression fitting, flexible rubber tubing was attached to allow for air flow down into the sludge.

The electrodes were again constructed of platinum and iron. Both electrodes were 254mm long and 0.508mm in diameter. The shapes were similiar elongated coils to provide equal surface area thus preventing unequal potential. From the exposed tip of each electrode, above the compression fitting, 16 gauge copper wire was connected to the electrode. The two copper wires were then connected to an electrometer to complete the external circuit of the cell. From the electrometer a strip chart recorder was connected to record the fuel cell's continuous output.

FUEL CELL SETUP

Further experiments were performed with activated sludge. The cell was prepared by sterilizing and the salt bridge poured into place. The electrolyte at the initial concentration was prepared for the anaerobic organism and placed in the cathode compartment. Desulfovibrio desulfuricans was then inoculated into the compartment and then outgassed with filtered nitrogen gas for five minutes at the same flow rate of two cubic feet per minute to remove oxygen. In place of the algae, activated sludge was added to the half-cell. 25 C air at a flow rate of 5 cubic

feet per minute was bubbled into the sludge from bottom to top as in a normal digester. After several minutes of established air supply, the electrometer was switched on along with the recorder. Data were taken and the experiment was performed six times. The activated sludge sample was obtained from a waste treatment facility in Wayne, New Jersey.

The activated sludge sample was kept viable by inoculating a 14 liter fermentor and supplying with air, nutrients and agitation. The sludge was maintained in this fashion for the life of the trials.

RESULTS

BIOCHEMICAL CELL TRIALS

The first group of experiments were performed to dupilcate N. Alexander's(1) work. In addition this group was comprised of two sets of experiments, one with the half cell compartments uninoculated and the other set with the compartments inoculated with micro-organisms. This uninoculated cell data collection presented a basis for determining the net electrical output of the fuel cell.

The experiments with uninoculated cells are shown in charts one thru six. Each trial was performed with the fuel cell immersed in a in a controlled temperature bath set at 37 C. In these six trials no microorganisms were present in either half cell. The concentrations of the media components for these uninoculated cell trials are shown in Tables I and III.

Chart 1 shows a trial time of 25 hours with a maximum cell output of 16mv, approximately 4 hours after the electrometer was turned on. Approximately 10 hours later, the cell output dropped to a constant 10mv level.

Chart 2 was different from Trial 1 in that a sustained output of 20mv was obtained 5 hours after initiation. The cell maintained that level for 3.5 hours before dropping back down to a 10mv level for the last 14.3 hours of operation.

Chart 3, represents unusual behaviour in that the cell output rose to a 15mv level 3 hours after the load bearing device was turned on, maintained that level for another 4 hours and then climbed to a 20mv level. The cell continued to produce an output of 20mv for 2.5 hours and then dropped off to a level of 16mv for a period of 8 hours. 21 hours after the start, the cell output leveled off to 10mv for the last 3.5 hours.

Chart 4 results indicate that the cell reached a peak level of 16mv in the first 4 hours of operation. The cell output then declined, and leveled off at 10mv 5 hours later. For the next 8 hours of operation the potential remained at 10mv only to drop down to a level of 8mv for the last 7 hours of measurement.

Chart 5 showed an output of 22mv, 8.5 hours into the experiment. The 22mv level remained for the next 1.5 hours, and then descended down to an 11mv output for the last 13.5 hours of operation.

The last uninoculated trial, experiment 6, shows a step-wise pattern as the output increased to about 16mv in the first 4 hours, and stayed at that output for 3 additional hours and then increased to approximately 20mv after 10 hours. At the end of 25 hours the measured output level was 8mv.

The uninoculated fuel cell maximum output, averaged for the six trials was 19.0mv, as shown in Table IV. This

group of experiments shows that a background voltage of 19mv exists in the electrolyte solutions without micro-organisms.

A review of Table XV shows the fuel cell output for an uninoculated cell at the initial media concentration. The six trials at this media concentration are tabulated at hourly intervals along with the average and standard deviation. A detailed examination reveals that the data fits a normal distribution curve. The highest standard deviation occurs in the hours 10 through 13 which represents the time frame where data differ the most. The data in hours 10-13 indicate a wide displacement around the average. There is a large spread from the mean in the 14-19 hour time frame also. This 5 hour time period represents the declining section of each trial, down to the constant output level.

Then the electrical potential of inoculated cells was determined. This grouping was performed with a nutrient concentration identical to charts one through six. The half cell compartments were prepared with media and then inoculated with its respective microorganism.

Chart 7 reveals an output level of 50mv, 5 hours after the inoculation. The fuel cell remained at this level for 3.5 hours before rising to a level of 60mv, 2.5 hours later. 15 hours later, the measured level had descended to an output of 40mv.

Chart 8 shows a similiar pattern except that a level of 50mv was reached 7 hours after inoculation. This 50mv level lasted for 2 more hours and then peaked at 60mv, only to drop almost immediately back down to a level of 50mv for the remaining 14 hours of measurement.

Chart 9 had a similiar rise to 50mv after inoculation in 7 hours, remained constant at 50mv for 3 hours and then climbed to 54mv. A decline back down to 50mv lasted for 6 additional hours with a slight drop to 40mv after 25 hours of observation.

Chart 10 shows no step up profile as in trial 9. The peak output of 62mv was measured 8 hours after inoculation, and that level was maintained for 1.5 hours. The voltage level then descended to 40mv 5.5 hours later. The final level of 30mv was measured after 24 hours of operation.

Trial 11 reflects a steep incline to 60mv at a constant rise over the first 11 hours of cell operation. The level then dropped off to 48mv and remained constant at that level for the remaining 8 hours of measurement.

Chart 12 indicates a rise to 56mv, 12 hours into the experiment. The potential remained at the 56mv level for 3 additional hours and then steadily declined over 9 hours to a final output level of 40mv.

This group of six inoculated trials revealed an average maximum output of 59.0mv as shown in Table IV. In comparing the biological activity of the microorganisms,

for the initial media concentration in the fuel cell, an average output of 19mv and 59mv for the uninoculated and inoculated cells respectively, can be determined. This indicates a net output potential of 40mv. Further comparison between the sets of experiments show that the uninoculated trials have an early peak electron transfer (within 4 hours of startup), while the inoculated trials produce peak potential at approximately the seventh hour.

Table XVI for inoculated trials at the initial concentration shows greater variance in two separate time frames as compared to the uninoculated trials. In time frame, hours four through six, the sample data is the highest for these trials. In the final hours of the trials, 22-24, the variance again is quite high. A review of the data within a normal distribution curve shows that at hour 5, some data are outside the curve. This indicates a degree of uncertainty of reproducibility for this time frame in the trials.

ELECTRICAL OUTPUT VARYING MEDIA CONCENTRATION

The next group of experiments were peformed to determine the effect of an increase in nutrient concentration on the electrical output potential. The nutrient concentration was modified from the original concentration to levels of 10X, 100X, and 1000X, and the fuel cell output measured. For these three phases of

evaluation all other variables were held constant.

10 FOLD MEDIA CONCENTRATION

Phase I represents a 10 fold increase in the media concentration. The electrolyte solutions as prepared, are shown in Tables V and VI for the microorganisms. The first six experiments were operated with both half cells uninoculated. As mentioned previously, this evaluation without microorganisms presents the background voltage of the electrolyte solutions less any biological activity.

Charts 13-18 show the output potential at this new media concentration with uninoculated cells. Each experiment was performed with the fuel cell immersed in a controlled temperature bath set a 37 C.

Chart 13 shows an immediate step change in output up to 10mv in 2 hours. The output remained at 10mv for the next 2 hours and gradually increased to 22mv 4 hours later. For approximately 9 more hours the cell sustained an output of 22mv before dropping back down to 10mv after 25 hours of operation.

The next experiment, trial 14, is different in that within 3.5 hours the electrical output had reached 20mv. The cell maintained the 20-22mv output for the next 13 hours before declining back down to 10mv for the last 7 hours of operation.

Chart 15 is similiar to the previous experiment in that there is a steady rise to maximum output without a

step change. In this particular trial , however, it takes almost 8.55 hours for the cell to reach an output level of 20mv. The potential remained at 20mv for the next 8 hours and then dropped off rapidly to 10mv. Chart 15 represents a lag of 5 hours, as compared to Chart 13, in reaching maximum output level.

Trial 16 is somewhat like chart 14 in that a slight step change is present. Three hours after initiation the cell output reached a level of 16mv. The data shows a very slight rise over the next 4 hours before climbing again to a level of 24mv. Approximately 3.5 hours of continous 24mv output are evident, before the output descended back to a 10mv level. For the last 7 hours of operation the cell output remained at 10mv.

The next experiment, Trial 17, revealed a trend similiar to that of Chart 15. A large step increase to 20mv occurred in 4 hours of cell operation. The next 10.5 hours represent a sustained output of 20mv followed by a gradual decline to 10mv over a 10 hour period.

The final uninoculated cell experiment, at the 10X media concentration, is something like trials 14 and 15. It took 9.5 hours before reaching the maximum output of 20mv. Once at 20mv, the electrical potential remained constant for approximately 9 hours with a quick step down to 12mv in the 5 remaining hours of operation.

For the six trials with a 10 fold media concentration

increase, the average uninoculated maximum output was 22mv, as shown in Table VII. In comparing the average maximum output of these trials, to the average maxiumum output at the initial media concentration, there is a 3mv increase.

The next data analysis covers the 10X media concentration increase for the uninoculated trials. For the six uninoculated trials the statistical data are shown in Table XVII. The standard deviation for all time periods is higher than for the same trials at the initial media level. However the data fits within a normal distribution curve. The higher standard deviation represents points further from the mean.

The next six trials represent the biochemical fuel cell with both half cells being inoculated with their respective microorganisms at a 10 fold media concentration increase. These experiments were performed to determine the net potential of the fuel cell at the increased media concentration.

Chart 19 is the first inoculated trial at the new media concentration. The first 10 hours after inoculation show a constant gradual rise to 68mv with no step changes. After reaching an output of 68mv the cell maintained this output for approximately 8 hours before descending. The potential then step changed down to 60mv and remained at that level for the first 7 hours of operation.

The next inoculated trial, Chart 20, shows a similiar

pattern. There is a steady rise, after inoculation, up to a 72mv output over a 12 hour period. The cell potential then remained constant for 7 hours at 72mv before stepping back down to an output level of 59mv. The cell output continued for 5 hours at the 59mv level.

Trial 21 follows the same pattern as the two previous trials with the inoculated cells having a 10 fold media concentration. The output climbed steadily for 12 hours to a level of 69mv. The potential however remained constant at 69mv for only 3 hours before decreasing. Over the last 10 hours of data collection, the cell potential slowly descended from 69mv to 52mv where it remained constant for the last 3 hours of operation.

The next inoculated trial is typical of the first three trials at this media concentration. There is a positive slope up to a 70mv output over an 11 hour time frame. The cell discharged at the 70mv level for approximately 4 hours before declining down to a 54mv level. The 16mv descent took 10 hours.

Experiment 23 shows a pattern similiar to the other trials in that there is a positive slope upward over an 11 hour period to a 72mv level. The measured potential in this case remained at this high output for 6 hours, before taking the pattern-like descent down to a lower level of 58mv. The decline in voltage, from 72mv to 58mv, occurred over a 6 hour time frame, before settling out at 58mv.

The last trial, 24, at the 10 fold media concentration with both half cells inoculated is different than the others in this group. The steady rise in output, after inoculation, in 12 hours to 72mv is typical. The variation however, is that after remaining at a 72mv output level for 3 hours, there is a gradual descent without a step down function. The cell steadily declined from 72mv to 50mv.

This group of inoculated fuel cell trials, at a 10X media increase, showed an average maximum output of 71mv. In comparison, for the uninoculated and inoculated fuel cell at this media concentration, an output of 22mv and 71mv respectively, a net potential of 49mv can be calculated. Further comparison between the inoculated cells only, at the initial and 10 fold media concentration, indicates a 4 to 5 hour time lag before reaching maximum output. Table VII represents the maximum measured output for each uninoculated and inoculated trial. Figure I shows the comparison of media concentration versus fuel cell output.

Table XVIII represents the calculated average and standard deviation for the 10 fold inoculated fuel cell trials. In comparison of the standard deviation for the initial and 10X media concentration trials, the 10-fold data are more close to the mean. However, in hours two through nine, which is 38% of the fuel cell time cycle, the data are outside a normal distribution curve.This indicates

lower repeatability in that time frame. By comparison only one hour period in the initial media concentration is outside a normal distribution.

100 FOLD MEDIA CONCENTRATION

The next twelve experiments were the second phase in evaluating the effects of an increase in nutrient concentration on the fuel cell output potential. This second phase also held all variables constant except that the nutrient concentration was increased to 100X the concentration of the first set of experiments. The media concentration for these experiments is shown in Tables VIII and IX.

The first six experiments, at a 100X media concentration, were performed with both half cell compartments uninoculated. As in the previous series of trials, the uninoculated half cell compartments are connected and the electrical potential across the salt bridge is measured. This is to determine the background voltage of the electrolyte solutions without biological activity.

Chart 25 is the first experiment in six where a 100 fold increase in media concentration is evaluated. After startup of the measurement/recording devices the potential climbs gradually in 10 hours to a level of 22mv. The output remains fairly constant at the 22mv level for the next 6

hours before a slow descent down to 10mv. The last 3 hours of cell operation show a constant 10mv output.

Experiment 26 is similiar to trial 25. After initiation, the output of the fuel cell climbs to 23mv in 10 hours. The voltage remains at about the 23mv level for 2.5 hours before drifting down to a 10mv output 20 hours after startup. For the 1st 5 hours of operation the cell output remained at the 10mv level.

Chart 27 is quite different from the previous two experiments, in that the ascent is faster. After 7 hours of operation, the fuel cell output has reached its maximum level of 22mv. As in the previous two trials, however, the output remains fairly constant for 8.5 hours at the 22mv level. A slow descent down to 10mv occurs 8.5 hours after startup, and remains at the 10mv level for the last 5 hours of observation.

Trial 28 reflects a normal uninoculated cell output at 100 fold media concentration as compared to charts 25 and 26. There is a gradual rise in 10 hours to a 22mv output, before a descent back down to a constant 10mv potential. The cell produced a 22mv output for 6 hours before declining. The last 7 hours of observation reflect a change in voltage of 12mv, with the cell dropping to 10mv from a level of 22mv.

Uninoculated trial 29 is somewhat different than the previous trials altogether. The potential increases in

about 10 hours to a 22mv level, similiar to the other experiments, however, there is a second step downward in the remaining hours of observation. After producing an output of 22mv for 3 hours, at hour 13, a decline begins. 4 hours later, at hour 17, the cell output is measured at 12mv. The cell output remains at the 12mv level for 5 more hours before dropping off to 10mv in the last hour. In comparison to the four previous trials, it seems as if significant changes in biological activity are occuring after reaching the maximum output.

Trial 30, the last uninoculated experiment at this higher media concentration, is also different. In the first 3 hours after startup, an output level of 4mv is held constant for 1 hour before suddenly increasing to a 22mv output at hour 7. The remaining time of experimentation shows a constant output of 22mv for 7.5 hours, then a step down to 6mv in 6 hours where the output remained constant for the last four hours.

In summarizing the output activity of the uninoculated 100X media concentration trials, only the maximum outputs were closely matched as shown in Table X. Various changes, before and after reaching the maximum output, seem to reveal periods of stagnation.

A review of the 100 fold uninoculated media trials output in Table XIX shows low standard deviation. Actually, there are two periods of low deviation throughout the time

frame. Time periods representing hours one through three and 9-13 have a standard deviation less than 1.00. The other two time periods with a higher standard deviation are almost equal in value. All of the data for these six uninoculated trials are again within a normal distribution curve.

The next experiments involve the inoculation of both half cell compartments at the 100X media concentration. Again the media concentration, as shown in Tables VIII and IX, were used in conjunction with their respective microorganisms. Trials 31-36 represent the inoculated 100 fold media concentration experiments.

Experiment 31 shows a sharp rise up to 80mv in 11 hours after inoculation. For the next 5 hours, the voltage measured remained constant at 80mv. At hour 16, a descent down to 60mv in 4 hours is evident. The cell output remained constant at the 60mv level for the remaining hours of observation.

Chart 31 shows a similiar pattern to the previous experiment. There is a pronounced voltage rise in the first 11 hours of operation to 80mv. The measured output fluctuates around the 80mv level for the next 5 hours before a downward trend is seen. The step down of 20mv, to the 60mv level, occurs over approximately 4 hours before levelling off to a constant 60mv for the final 4 hours of observation.

Trial 33 reveals a measured cell output that is different than the previous two trials. The sharp incline to 80mv is evident in 11 hours after initiation, however once the maximum output of 80mv is reached the voltage remained constant for 7 hours, as compared to 5 hours for the previous trials. The descent pattern is similiar to the other trials except that it took only 3.5 hours to drop to a 59mv level. The measured voltage remained constant at this level for the 3 final hours of observation.

Experiment 34 reflects a pattern similiar to trials 31 and 32, with the usual initial rise and downward trend. The maximum output of 78mv was reached in 11 hours and remained constant for 5 hours at that level. The downward trend from 78mv to 54mv occurred in 5 hours and remained at the 54mv level for the last 5 hours of data collection.

Chart 35 deviates from the previous experiment's profile. This experiment begins with a steady increase in 12 hours to reach a level of 82mv but remains at this level for only 3 more hours. A slight step downward to 80mv occurs in 1.5 hours before descending again to a level of 60mv in 5.5 hours. The cell remained at the 60mv level for an additional 2.5 hours. This particular experiment shows two steps downward after reaching the maximum output of 82mv.

The last trial in Phase II, for an inoculated 100X media concentration level, indicates an experiment similiar

to Trial 33. The pronounced rise in 12 hours to a maximum output of 83mv is evident. As in trial 33, the output remained fairly constant for the next 7 hours at the 83mv level before beginning its downward trend. The descent down to 60mv occurred in 4 hours and remained at that level for the last 2 hours of measurement.

For these inoculated trials, the average maximum output was 81mv, as shown in Table X. As experienced in the uninoculated group at the 100X media concentration, various trends before and after reaching the maximum output are evident. While four of the six trials show similiar activity the other two show longer periods of activity at the maximum output. Plotting the summarized data in Table X, on semi-logarithmic paper, Figure V represents the biochemical fuel cell output in millivolts versus media concentration. As compared to the two previous media concentration levels, there is a slight increase in voltage output. The net potential at 100 fold media concentration, as determined by the difference between 24mv and 82mv for the uninoculated and inoculated cells respectively, is 58mv. For the initial media concentration the net potential was determined to be 40mv and at a 10 fold level, a 50mv net potential was produced. This plot reveals a 20% increase at the 10 fold media level and a 45% increase in fuel cell output for the 100 fold media level, as compared to the initial media trials.

Table XX is the data analysis of the inoculated fuel cell trials at 100X media concentration. A review of these shows a low standard deviation but for one period of high value. Hours 18 to 21 have a standard deviation higher than the rest of the population. Thus the individual data points are a little scattered at these time intervals. All of the data for these trials fit within a normal distribution curve.

1000 FOLD MEDIA CONCENTRATION

Phase III represents the final comparison of media concentration versus fuel cell output. Twelve trials were made. The first six with both half cells uninoculated and the other six with both half cells inoculated. The media concentration for these trials was increased 1000X over the initial fuel cell levels. The media concentrations for these experiments are shown in Tables XI and XII.

Trial 37 is the first uninoculated experiment at the 1000 fold media concentration. Again neither half cell was inoculated with microorganisms and the electrical potential of the electrolyte solutions across the salt bridge was measured. Upon initiation of the measuring devices there is a gradual increase in potential to 24mv in about 10 hours. The cell output remained at this level for 5 hours before a step down began. 20 hours into the trial the cell output finally reached a steady lower output level of 10mv for the last 4 hours of observation.

The next uninoculated trial was similiar to the previous experiment, in that there is a gradual rise over 10 hours to the maximum output level of 24mv. After a 6 hour duration at the 24mv level a descent occurs. The sudden downward step change takes only 3 hours, as compared to 4 hours for the previous trial, to a final level of 10mv. The fuel cell output remained at a constant 10mv level for the last 5 hours.

Chart 39 is different than the two previous trials in that the rise in 9 hours to a level of 23mv is slightly faster than in the other two trials. The duration at 23mv is only 2.5 hours, and the descent is slower, in that it takes 8 hours to level off to around the same 10mv level. There is no quick downward step change, just a gradual descent. One final note of difference is that the last two hours of observation show a small positive step upward to 12mv, which the other two trials had not produced.

For chart 40, the first half of the time frame shows a similiar pattern to the first two trials, a 10 hour slowly increasing output to 25mv. The duration at the 25mv level is short lived as a downward descent is evident immediately, with a gradual tapering down to 20mv in 7 hours. Another quick descent, from 20mv to 11mv output, in two hours is also apparent. However, as in the previous experiment there is a sudden step up to 12mv in the final 3 hours of observation.

Chart 41 is somewhat typical of this uninoculated group. A gradual rise in 12 hours, instead of 10, to an output of 26mv is evident. A unique phenomena occurs for this experiment in that no step changes are evident, just a gradual levelling off. The output slowly descends over the last 13 hours of observation before the last one hour of constant output at 6mv is observed. No other trial in this group has similiar characteristics.

The last uninoculated trial, 42, in this 1000X media concentration group exhibits trends similiar in nature to trials 37-40. A gradual rise to a maximum output of 25mv in 10 hours, with a 2.5 hour time duration at the 25mv level before a gradual descent is observed. Descent takes 7 hours to reach a constant output level of 10mv for the last 4 hours of cell operation.

Table XIII summarizes the average maximum output for the six uninoculated trials with the 1000 fold media concentration. These experiments reveal even more changes for uninoculated fuel cells than the others at lower nutrient concentration levels. No step changes downward and sudden upward surges at the end of the trials indicate unsteady state conditions.

Table XXI shows the analyzed data for the six uninoculated trials at the 1000-fold media concentration. The table shows a very low standard deviation throughout the time period. In comparison to the three previous

uninoculated groups this data group have the lowest standard deviation. This indicates that the 1000-fold trials have the highest degree of confidence, greater than the other three groups. All the data fit within a normal distribution curve.

The next six trials reflect a 1000 fold media concentration fuel cell with both half cells inoculated with their respective microorganisms. These experiments were performed to determine the net potential at this media concentration when compared to the previous uninoculated trials.

Experiment 43 shows a sharp rise in 13 hours to an output level of 88mv. This 88mv level was held constant for approximately 1 hour before a gradual decline is seen over the next 4 hours to the 81mv level. The downward slope then increases as a drop of 17mv is seen in 4 hours from 81mv to 64mv. The 64mv output level held constant for the last 3 hours of cell operation.

Chart 44 is the next experiment in this 1000 fold media group. This trial shows a more pronounced step change. It took 11 hours to climb to an output level of 87mv where this level held for a duration of 5 hours. At the end of the 87mv level the output dropped quickly to a 72mv level before gradually tapering off to 64mv. As compared to the previous trial, chart 44 is similiar except that the downward change is more pronounced and occurs

earlier in the trial.

Experiment 45 is similiar to that of trial 44 in all aspects. The gradual rise to the maximum output of 87mv occurs in 12 hours. A sustained output of 87mv for 6 hours is evident. A gradual downward slope to an output of 68mv which the fuel cell discharged for the last 5 hours.

The next inoculated trial shows peculiar data in the area of sustained high level output. In chart 46 there is the prevalent gradual rise in 12 hours to an output level of 92mv. For the next 5 hours there is a slight up and down shift between 92 and 88mv before settling out at 90mv. Another similiar trend is evident after the high level output. The slope of the trial is negative for 4 hours, down to an output of 66mv where the discharge voltage seems to level off for the remainder of the experiment.

Trial 47 exhibits the same pattern as chart 46. In the high level output a seesaw effect is seen. The gradual rise to 90mv is established in 1 hours and the up and down pattern takes 3 hours, going from 90mv down to 87mv and back up to 90mv. The output remained at the 90mv level for 1.5 hours more before descending. The step change in this experiment is more pronounced, in that it takes only 3 hours to quickly drop from 90mv to 68mv. There is however, a longer time frame, of 6 hours, where the final output hovers around 67mv.

The last experiment in the 1000 fold inoculated media

concentration group is chart 48. This trial represents a pattern similiar to charts 43-45. A gradual rise in 13 hours to 90mv, with a sustained 90mv output for 5½ hours, in 4 hours. Then a downward step change from 90mv to 73mv occurs. The final 5 hours of operation show an ever slight decrease down to 68mv before data collection ceases.

Table XIII lists the maximum output level for the six inoculated 1000 fold media concentration trials. The average of these six experiments is 89mv. In comparing the output data, the unsteady state conditions of electrical output is prevalent mostly in the high level discharge area Large fluctuations occur once the maximum output is obtained for all six trials.

The data in Table XXII reveal two portions where the standard deviation is high. For hours 7-10 and 18-20 the standard deviation is 5.0 or higher up to 7.5. This indicates a scattering of data in these time periods for the six experiments. In comparison to the other inoculated groups at various media concentrations, the initial and 1000X media groups closely resemble one another.

Based on the average maximum output of all 48 trials for the uninoculated and inoculated experiments, Figure VI was prepared. Figure VI shows the comparison of the fuel cell output versus the media concentration. For the uninoculated group it can be shown that for a 1000 fold media increase the average fuel cell output is 25mv. This

is a 39% increase in cell output for the 1000 fold media concentration as compared to the initial media concentration for unincoluated fuel cells. Comparing the output at 100 fold to the 1000 fold level, there is only a 8% increase, with the potential rising from 23mv to 25mv.

In summary most data for the 48 experiments fall within normal distribution curves. Only the initial and 10X media levels were outside the distribution curve with the 10-fold group having the most time periods out of the norm. Thus the degree of confidence is below 0.90 for the entire data population. However the data is reproduceable and only 1% of the data are off base. As for determining the most confident trials, the 1000X uninoculated media group is probably the most reproduceable due to the low standard deviation. The 100X media level in the inoculated group is the second best group for duplication.

In comparing the inoculated fuel cell average output at the various media concentrations a different viewpoint is evident. For the initial media concentration the average maximum output was 58mv. For the 1000 fold media level the average output was 89mv. This is a 51% increase in output between the two media levels.

In determining the net potential of the cell at the various media concentration, Figure VI data were used. Viewing the plot the following is evident; at the initial concentration a 40mv net potential is available, at a 10

fold media increase the net potential rises to 49mv, for a 100 fold media level the output increases to a 58mv net potential and at a 1000 fold media concentration, the net potential reaches a 64mv level. In comparing the initial media concentration net potential to the 1000 fold media concentration net potential there is a 58% increase from 40mv to 64mv.

A final comparison is shown in Figure VII. Fig. VII is a log-log plot of net potential versus media concentration for both inoculated and uninoculated cells. It can be seen for both the groups across the media concentrations that almost a straight line is drawn. The regression coefficient that indicates the degree of confidence for each group of data is 0.72 and 0.77 for uninoculated and inoculated fuel cells respectively.

ACTIVATED SLUDGE FUEL CELL TRIALS

The second application of the fuel cell was one in which the microorganisms were changed. This change involved replacing the one half cell containing the algae, Scenedesmus quadricauda, with activated sludge from a waste treatment facility. The cell otherwise remained the same as used in the previous trials, including the salt bridge and the Desulfovibrio desulfuricans in the other compartment. The purpose of these experiments was to determine the electrical output of the cell, using the activated sludge.

Experiments with the inoculated anaerobic half cell and the other half cell with activated sludge are shown in charts 49-54. Each trial was performed at an average bath temperature of 37 C. In these trials, inoculated Starkey's Desulfovibrio media, as shown in Table III, was used along with the activated sludge sample taken from a local waste treatment facility.

The first trial, experiment 49, shows a steady rise in 10 hours to an output level of 38mv. The cell output remained at the 38mv level for about 3 hours. The output continued, fluctuating up and down for about 12 hours before a slight decline down to about 35mv. No step change was evident in the cell output after reaching the maximum level.

Chart 50 is quite similiar except that in the initial rise, there seems to be an increase in activity 4.5 hours after inoculation. At this point the slope of the curve rises more sharply up to 37mv at the 9 hour mark into the trial. A steady 3 to 3.5 hour output at 37mv is evident. At this point the cell output fluctuated for the next 12 hours ending at a 34mv level after 24.5 hours of operation.

Chart 51 output closely resembles trial 50. In the initial rise, after cell inoculation, there are two evident steps before reaching a maximum output level. The first step, at hour 5 rose from 15mv to 25mv. the next step

occurred at hour 7.5, where the output climbed from 25mv to reach a level of 38mv at hour 10.For the 9 hour period from hour 10 to hour 19, the cell output is fairly smooth between 38 and 40mv. From hour 19 on, there is a slight decline that falls to 36mv at hour 24.

Trial 52 is similiar to experiment 49. A large gradual rise occurs after cell inoculation to an output level of 37mv at hour 11. From hour 11 to hour 20 the cell output was constant at the 38mv level. Then abruptly 0.5 hours later, the cell output rose slightly to 40mv, the highest for the trial. For another 3 hours the 40mv output remained constant before sloping back down to 37mv for the last 2 hours of operation.

Experiment 53 follows no set pattern. However like chart 51, this trial did have two changes. In the first 9 hours, the cell output rose to 30mv, held constant at 30mv for 2.5 hours in the beginning, before rising again to 40mv in 5 more hours. The next 7.5 hours reveal a constant output at the 40mv level before descending down to 35mv in the last 2 hours of the trial.

The last experiment in the activated sludge series, trial 54, is totally different from all the others. There were no evident step changes or extended periods of constant output. This trial is unique in that it took almost 19 hours, out of the 25 hours of operation, to reach the maximum level of 39mv. Once that level was reached,

the last six hours show an irregular pattern before declining down to the 37mv level.

For these six activated sludge trials, the average fuel cell output was 39mv. There were no significant trends that tie the experiments together, however, some similarities are evident to a small degree. Table XIV lists the average output data for the trials. A comparison of the activated sludge trials with its algae counterpart shows an average maximum output of 38mv for the sludge trials and 55mv for the algae trial at the same media concentration. This reveals a decrease of 31% in fuel cell output using the activated sludge for the aerobic half cell as compared to the algae half cell.

For the activated sludge fuel cell trials the standard deviation is well within the values obtained for the non-sludge experiments. The standard deviation values in Table XXIII reveal a range of 0.86 to 4.88. Curiously, a time period of high standard deviation occurs during the hours 6-11 only, much like the 1000X inoculated trials. Additionally, this activated sludge data fit a normal distribution curve with a 0.90 confidence level.

The next comparison, involves the calculated net potential of the activated sludge trials. The net potential was calculated by subtracting the measured average uninoculated fuel cell output (19mv) from the average activated sludge cell output (39mv) to obtain a

value of 20mv. In comparing the net potential of the algae inoculated fuel cell with the activated sludge cell, the algae fuel cell has a net output of 40mv as compared to 20mv for the activated sludge output. This is a 50% decrease in net fuel cell output.

CONCLUSIONS

In review of the results presented, it is clear that this biochemical fuel cell is a viable design for producing electricity. In addition, the experimentation showed that activated sludge from a waste treatment facility, produced electricity but at a lower level. Finally, increases in the concentration of nutrient components produce direct increases in electrical output.

The data verifies the biochemical fuel cell work of Sutton et al(12) by having similiar voltage levels for approximately the same periods of time, using a salt-bridge type of fuel cell. A review of the literature, reveals no evidence of experimentation on the effects of nutrient concentration on biochemical fuel cell output. Further analysis of previous work indicates that the successful application of activated sludge in a biochemical fuel cell is a new venture.

A discrepancy appears in the literature based on the verification of Sutton et al(12) by these experimental results. Davis et al(3) reports an output voltage of 0.2 to 0.58 volts as compared to the verified voltage range of 0.02 to 0.09 volts.

The experiments utilizing activated sludge show a logical starting point for further development of biochemical fuel cells. Although the measured electrical

output, as compared to the non-sludge fuel cell, is lower, several parameters must be considered. Conditions of pH, lower nutrient concentration, level of oxygen and less favorable enviromental conditions may explain the activated sludge fuel cell's lower output. Alternatively, the application of an activated sludge biochemical fuel cell must be pursued. Consider a fuel cell consisting of two 10,000 gallons tanks connected by a salt bridge. Large electrodes with a surface area of 35,000 sq. ft., a ratio of 0.00058 sq. ft./ml of solution, are placed in the tanks. This is the same volume to surface ratio of the experimental fuel cell. With activated sludge in one tank and Desulfovibrio desulfuricans, or a similiar reducing organism in the other an estimated 715 volts could be obtained. Thus a waste treatment facility could become all or partially energy self sufficient.

Future work should first resolve the noted discrepancy which would involve extended duplication of these results. Additional experimentation involving the activated sludge fuel cell performance parameters would follow. As with the non-sludge trials, the effects of nutrient concentration changes on the activated sludge fuel cell output should be studied. In addition, the effects of an increase/decrease in oxygen supply and the pH levels must be evaluated along with the effects of temperature. Lastly, the concept of

the cell configuration including the electrode surface area and the salt bridge are further avenues to pursue, before the biochemical fuel cell becomes a common energy practice.

APPENDIX 1

LIST OF MATERIALS

CHEMICAL	MANUFACTURER
Potassium orthophosphate, mono-h	Fisher Scientific
Potassium orthophosphate, di-h	Fisher Scientific
Magnesium sulfate, heptahydrate	MCB
Ammonium chloride	Fisher Scientific
Anhydrous sodium sulfate	MCB
Ferrous ammonium sulfate	J.T.Baker
Sodium lactate	Fisher Scientific
Peptone	Difco Laboratories
Beef extract	Difco Laboratories
Yeast extract	Difco Laboratories
Potassium nitrate	Fisher Scientific
Iron sulfate	MCB
Boric acid	J.T.Baker
Maganese sulfate	J.T.Baker
Zinc sulfate	Fisher Scientific
Copper sulfate	Mallinckrodt
Glucose	Corn Products Co.
Agar	Difco Laboratories
Sodium chloride	Mallinckrodt

APPENDIX 2

LIST OF EQUIPMENT

MAJOR EQUIPMENT	MANUFACTURER
"U" shaped casing with closures	Hand-crafted
Autoclave	Amsco Model 2053 Vagamatic
Electrometer	YSI Model 81A
Strip chart recorder	Coleman Model 56
Constant temperature bath	Precision Model 270
Gas Pak jar	BBL GasPak 150
Fluorescent lamp	Ledu Corp. Model S-284
Incubator	Imperial II Lab-Line Model 600
MINOR EQUIPMENT	
Glassware 100ml beaker 500ml beaker 2000ml beaker	Pyrex
Tubing	Chemfluor
16 gauge copper wire	B & S Wire
Platinum wire	Engelhard

APPENDIX 3

LIST OF TABLES

TABLE I

SCENESDESMUS QUADRICAUDA MEDIA

The following compounds in the amounts designated per liter of solution were utilized in conjunction with Scenedesmus algae:

Compound	grams
KNO_3	1.0
KH_2PO_4	0.135
$MgSO_4 \cdot 7H_2O$	0.500
$FeSO_4$	0.02
micronutrients	1.01 mls

This media was adjusted with sodium hydroxide to a pH of 7.0. The micronutrients were composed of the following compounds per liter of solution:

Compound	grams
H_3BO_3	50.0
$MnSO_4$	13.0
$ZnSO_4$	1.0
$CuSO_4$	0.5
$(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$	0.5

The micronutrients were adjusted to a pH of 7.0.

TABLE II

ALEXANDER'S AQUEOUS ELECTROLYTE (AEE)

DESULFOVIBRIO DESULFURICANS MEDIA

The following compounds in the amounts designated per liter of solution were utilized in conjunction with Desulfovibrio desulfuricans bacterium:

Compound	grams
K_2HPO	0.5
KH_2PO_4	1.0
$MgSO_4 \cdot 7H_2O$	1.0
NH_4Cl	1.0
Na_2SO_4	1.0
$Fe(NH_4)SO_4 \cdot 6H_2O$	0.1
Sodium lactate	4.0 mls.

TABLE III

STARKEY'S DESULFOVIBRIO MEDIA

The following compounds in the amounts desginated per liter of soluttion were utilized:

Compound	grams
Peptone	5.0
Beef Extract	3.0
Yeast Extract	0.2
$MgSO_4$	1.5
Na_2SO_4	1.5
Ferrous ammonium sulfate	0.2
Agar	15.0
Glucose	5.0

Adjust to pH of 7.4

For liquid media delete the agar.

TABLE IV

BIOCHEMICAL FUEL CELL POTENTIAL

INITIAL MEDIA CONCENTRATION

CHART	UNINOCULATED CELL OUTPUT, millivolts
1	16.0
2	21.0
3	20.0
4	16.0
5	22.0
6	21.0
	avg. 19.0

CHART	INOCULATED CELL OUTPUT, millivolts
7	60.0
8	59.0
9	54.0
10	62.0
11	60.0
12	56.0
	avg. 59.0

TABLE V

SCENEDESMUS QUADRICAUDA ELECTROLYTE

10X MEDIA CONCENTRATION

The following compounds in the amounts designated per liter of solution were utilized in conjunction with Scenedesmus algae to produce a ten fold increase in media concentration:

Compound	grams
KNO_3	10.0
KH_2PO_4	1.35
$MgSO_4 \cdot 7H_2O$	5.00
$FeSO_4$	0.20
micronutrients	10.1mls

This media was adjusted with sodium hydroxide to a pH of 7.0.

TABLE VI

STARKEY'S DESULFOVIBRIO MEDIA

10X MEDIA CONCENTRATION

A ten fold increase in Starkey's media concentration in amounts designated per liter of solution for Desulfovibrio desulfuricans bacteria:

Compound	grams
Peptone	50.0
Beef Extract	30.0
Yeast Extract	2.0
$MgSO_4$	15.0
Na_2SO_4	15.0
Ferrous ammonium sulfate	2.0
Agar	15.0
Glucose	50.0

Adjust to pH of 7.4

For liquid media delete the agar.

TABLE VII

BIOCHEMICAL FUEL CELL POTENTIAL

10X MEDIA CONCENTRATION

CHART	UNINOCULATED CELL OUTPUT, millivolts
13	22.0
14	21.0
15	20.0
16	25.0
17	21.0
18	21.0
	avg. 22.0

CHART	INOCULATED CELL OUTPUT, millivolts
19	70.0
20	73.0
21	70.0
22	71.0
23	72.0
24	72.0
	avg. 71.0

TABLE VIII

SCENEDESMUS QUADRICAUDA ELECTROLYTE

100X MEDIA CONCENTRATION

The following compounds in the amounts designated per liter of solution were utilized in conjunction with Scenedesmus algae to produce a ten fold increase in media concentration:

Compound	grams
KNO_3	100.0
KH_2PO_4	13.5
$MgSO_4 \cdot 7H_2O$	50.0
$FeSO_4$	2.0
micronutrients	101.0 mls

This media was adjusted with sodium hydroxide to a pH of 7.0.

TABLE IX

STARKEY'S DESULFOVIBRIO MEDIA

100X MEDIA CONCENTRATION

A ten fold increase in Starkey's media concentration in amounts designated per liter of solution for Desulfovibrio desulfuricans bacteria:

Compound	grams
Peptone	500.0
Beef Extract	300.0
Yeast Extract	20.0
$MgSO_4$	150.0
Na_2SO_4	150.0
Ferrous ammonium sulfate	20.0
Agar	15.0
Glucose	500.0

Adjust to pH of 7.4

For liquid media delete the agar.

TABLE X

BIOCHEMICAL FUEL CELL POTENTIAL

100X MEDIA CONCENTRATION

CHART	UNINOCULATED CELL OUTPUT, millivolts
25	24.0
26	24.0
27	23.0
28	23.0
29	22.0
30	24.0
	avg. 23.0

CHART	INOCULATED CELL OUTPUT, millivolts
31	81.0
32	82.0
33	82.0
34	76.0
35	83.0
36	84.0
	avg. 81.0

TABLE XI

SCENEDESMUS QUADRICAUDA ELECTROLYTE

1000X MEDIA CONCENTRATION

The following compounds in the amounts designated per liter of solution were utilized in conjunction with Scenedesmus algae to produce a ten fold increase in media concentration:

Compound	grams
KNO_3	1000.0
KH_2PO_4	135.0
$MgSO_4 \cdot 7H_2O$	500.0
$FeSO_4$	20.0
micronutrients	1010.0mls

This media was adjusted with sodium hydroxide to a pH of 7.0

TABLE XII

STARKEY'S DESULFOVIBRIO MEDIA

1000X MEDIA CONCENTRATION

A ten fold increase in Starkey's media concentration in amounts designated per liter of solution for Desulfovibrio desulfuricans bacteria:

Compound	grams
Peptone	5000.0
Beef Extract	3000.0
Yeast Extract	200.0
$MgSO_4$	1500.0
Na_2SO_4	1500.0
Ferrous ammonium sulfate	200.0
Agar	15.0
Glucose	5000.0

Adjust to pH of 7.4

For liquid media delete the agar.

TABLE XIII

BIOCHEMICAL FUEL CELL POTENTIAL

1000X MEDIA CONCENTRATION

CHART	UNINOCULATED CELL OUTPUT, millivolts
37	25.0
38	25.0
39	24.0
40	26.0
41	27.0
42	25.0
	avg. 25.0

CHART	INOCULATED CELL OUTPUT, millivolts
43	89.0
44	88.0
45	88.0
46	91.0
47	92.0
48	91.0
	avg. 89.0

TABLE XIV

ACTIVATED SLUDGE FUEL CELL POTENTIAL

INITIAL MEDIA CONCENTRATION

CHART	CELL OUTPUT, millivolts
49	38.0
50	37.0
51	39.0
52	40.0
53	40.0
54	39.0
	avg. 38.8

TABLE XV
STATISTICAL ANALYSIS
UNINOCULATED INITIAL CONC.

UNINOCULATED TRIALS
MEDIA CONC. 1X
CELL OUTPUT, millivolts

TIME,HRS \ TRIALS	1	2	3	4	5	6	AVERAGE	MINIMUM	MAXIMUM	STD DEV	VARIANCE
1.00	9.00	5.00	10.00	5.80	3.40	3.60	6.13	3.40	10.00	2.53	6.41
2.00	10.00	9.60	10.00	10.20	6.50	6.80	8.85	6.50	10.20	1.57	2.46
3.00	12.00	10.40	14.60	13.80	8.60	9.40	11.47	8.60	14.60	2.21	4.86
4.00	16.00	10.20	15.00	16.00	10.40	12.00	13.27	10.20	16.00	2.49	6.20
5.00	15.00	19.60	14.40	16.10	15.80	12.00	15.48	12.00	19.60	2.27	5.16
6.00	15.00	20.00	14.60	15.30	17.90	12.30	15.85	12.30	20.00	2.47	6.10
7.00	15.00	20.40	18.00	15.00	19.00	12.90	16.72	12.90	20.40	2.61	6.81
8.00	14.60	20.20	20.00	13.50	20.80	15.60	17.45	13.50	20.80	2.96	8.74
9.00	12.40	14.60	19.60	12.00	20.80	17.50	16.15	12.00	20.80	3.39	11.51
10.00	10.40	10.00	20.00	10.00	20.00	19.80	15.03	10.00	20.00	4.90	24.03
11.00	10.00	9.60	17.60	11.40	16.50	20.00	14.18	9.60	20.00	4.02	16.19
12.00	10.00	9.00	15.60	9.10	13.50	20.60	12.97	9.00	20.60	4.18	17.50
13.00	10.00	9.40	16.00	10.30	12.80	18.90	12.90	9.40	18.90	3.49	12.17
14.00	10.00	9.40	15.60	10.00	12.50	17.80	12.55	9.40	17.80	3.16	9.97
15.00	9.60	11.00	14.40	10.40	12.00	16.20	12.27	9.60	16.20	2.32	5.38
16.00	10.00	10.40	13.40	10.00	12.00	15.60	11.90	10.00	15.60	2.06	4.24
17.00	10.60	10.60	13.40	8.80	11.70	14.00	11.52	8.80	14.00	1.77	3.13
18.00	10.00	10.80	13.60	8.50	11.50	13.60	11.33	8.50	13.60	1.84	3.40
19.00	10.00	10.40	12.00	9.00	12.20	11.80	10.90	9.00	12.20	1.18	1.40
20.00	10.00	10.00	10.00	8.40	11.70	10.40	10.08	8.40	11.70	0.96	0.93
21.00	10.40	10.00	9.60	8.30	12.20	9.60	10.02	8.30	12.20	1.17	1.37
22.00	10.20	10.00	10.00	8.00	11.60	10.00	9.97	8.00	11.60	1.05	1.10
23.00	10.00	10.20		7.80	10.60	9.20	9.56	7.80	10.60	0.99	0.98
24.00	10.00	10.00		6.30	9.00	8.50	8.76	6.30	10.00	1.36	1.85
25.00						8.00	8.00	8.00	8.00		
26.00											
27.00											

TABLE XVI
STATISTICAL ANALYSIS
INOCULATED INITIAL CONC.

INOCULATED TRIALS
MEDIA CONC. 1X
CELL OUTPUT, millivolts

TRIALS	7	8	9	10	11	12	AVERAGE	MINIMUM	MAXIMUM	STD DEV	VARIANCE
TIME,HRS											
1.00	9.60	9.00	6.00	4.20	7.10	3.00	6.48	3.00	9.60	2.38	5.67
2.00	19.60	19.20	10.00	9.70	13.60	11.40	13.92	9.70	19.60	4.08	16.63
3.00	29.60	20.00	19.80	21.70	19.80	18.80	21.62	18.80	29.60	3.67	13.48
4.00	40.00	26.50	24.60	34.20	26.50	22.00	28.97	22.00	40.00	6.18	38.15
5.00	50.00	33.00	30.00	46.50	35.30	28.00	37.13	28.00	50.00	8.25	68.01
6.00	50.40	39.60	40.40	52.30	42.00	35.90	43.43	35.90	52.30	5.91	34.98
7.00	50.20	49.60	49.60	56.40	47.30	42.00	49.18	42.00	56.40	4.25	18.10
8.00	50.00	50.00	50.00	60.60	51.10	49.30	51.83	49.30	60.60	3.96	15.65
9.00	52.80	50.40	50.00	61.50	54.30	50.90	53.32	50.00	61.50	3.95	15.59
10.00	57.80	58.20	50.50	53.70	57.20	51.80	54.87	50.50	58.20	3.03	9.17
11.00	60.00	54.00	52.60	54.80	59.70	51.60	55.45	51.60	60.00	3.27	10.71
12.00	50.00	52.40	53.80	54.00	53.90	54.60	53.12	50.00	54.60	1.54	2.38
13.00	49.00	51.60	52.20	50.30	52.30	55.50	51.82	49.00	55.50	2.01	4.04
14.00	48.00	51.40	51.00	45.90	52.00	55.70	50.67	45.90	55.70	3.10	9.60
15.00	47.00	50.60	50.60	40.60	50.00	55.00	48.97	40.60	55.00	4.41	19.45
16.00	44.00	51.00	49.80	37.30	49.50	50.80	47.07	37.30	51.00	4.96	24.60
17.00	40.80	50.60	50.00	36.50	48.40	50.40	46.12	36.50	50.60	5.47	29.91
18.00	40.40	51.60	49.80	34.60	48.50	49.50	45.73	34.60	51.60	6.13	37.53
19.00	39.00	49.00	49.60	35.80	48.70	47.80	44.98	35.80	49.60	5.47	29.89
20.00	39.00	49.40	50.00	33.40	48.60	47.30	44.62	33.40	50.00	6.22	38.71
21.00	40.40	50.00	48.00	33.70	49.10	44.70	44.32	33.70	50.00	5.73	32.82
22.00	39.00	49.60	49.00	32.00	47.90	42.30	43.30	32.00	49.60	6.33	40.09
23.00	40.20	49.00	45.00	29.00	48.50	40.60	42.05	29.00	49.00	6.76	45.74
24.00	40.20	50.00	44.00	28.00	48.30	37.80	41.38	28.00	50.00	7.33	53.71
25.00	40.20		41.00				40.60	40.20	41.00	0.40	0.16
26.00	39.80						39.80	39.80	39.80		
27.00	41.00						41.00	41.00	41.00		

TABLE XVII
STATISTICAL ANALYSIS
UNINOCULATED 10-FOLD CONC.

UNINOCULATED TRIALS
MEDIA CONC. 10X
FUEL CELL OUTPUT, millivolts

TRIAL / TIME,HRS	13	14	15	16	17	18	AVERAGE	MINIMUM	MAXIMUM	STD DEV	VARIANCE
1	2.30	2.80	1.80	3.40	4.10	2.40	2.80	1.80	4.10	0.76	0.58
2	4.80	5.20	4.30	8.60	8.80	3.60	5.88	3.60	8.80	2.05	4.21
3	5.20	8.00	5.20	16.30	11.70	5.00	8.57	5.00	16.30	4.19	17.56
4	5.20	10.00	6.20	16.50	18.20	8.10	10.70	5.20	18.20	4.96	24.61
5	5.80	10.20	7.30	17.20	19.60	9.80	11.65	5.80	19.60	5.04	25.45
6	7.70	10.20	8.00	17.80	19.80	11.00	12.42	7.70	19.80	4.69	22.03
7	9.20	10.30	9.00	18.40	19.80	11.80	13.08	9.00	19.80	4.37	19.09
8	10.00	10.60	9.20	20.90	19.90	13.40	14.00	9.20	20.90	4.71	22.23
9	10.30	10.50	9.70	23.80	20.00	17.70	15.33	9.70	23.80	5.47	29.92
10	10.70	10.30	10.10	24.00	19.90	20.30	15.88	10.10	24.00	5.67	32.17
11	10.70	10.20	9.80	24.20	19.90	20.50	15.88	9.80	24.20	5.81	33.80
12	10.70	10.10	9.50	23.80	20.20	20.00	15.72	9.50	23.80	5.76	33.19
13	10.80	10.20	9.80	22.30	19.50	20.20	15.47	9.80	22.30	5.28	27.83
14	10.30	9.50	9.90	19.80	20.10	20.40	15.00	9.50	20.40	5.11	26.09
15	10.20	9.40	9.50	16.00	18.40	20.30	13.97	9.40	20.30	4.45	19.82
16	10.00	9.10	9.80	11.00	18.40	20.20	13.08	9.10	20.20	4.46	19.90
17	9.50	8.80	9.40	10.00	18.00	19.60	12.55	8.80	19.60	4.46	19.87
18	8.30	7.30	8.00	9.40	14.90	18.10	11.00	7.30	18.10	4.04	16.36
19	7.60	6.00	6.00	9.40	14.10	18.40	10.25	6.00	18.40	4.57	20.85
20	6.70	5.90	5.30	8.80	13.60	15.00	9.22	5.30	15.00	3.78	14.25
21	6.50	5.70	5.10	8.50	11.00	14.40	8.53	5.10	14.40	3.28	10.74
22	6.00	5.20	4.30	7.80	10.60	13.60	7.92	4.30	13.60	3.26	10.61
23	5.40	5.00	4.50	7.10	9.60	12.80	7.40	4.50	12.80	2.95	8.71
24	5.00	4.80			9.70	11.70	7.80	4.80	11.70	2.99	8.91
25											
26											

TABLE XVIII
STATISTICAL ANALYSIS
INOCULATED 10-FOLD CONC.

INOCULATED TRIALS
MEDIA CONC. 10X
FUEL CELL OUTPUT, millivolts

TRIAL	19	20	21	22	23	24	AVERAGE	MINIMUM	MAXIMUM	STD DEV	VARIANCE
TIME,HRS											
1	6.40	3.60	5.20	4.40	4.90	3.10	4.60	3.10	6.40	1.08	1.16
2	10.60	6.80	7.80	9.80	6.90	7.80	8.28	6.80	13.80	1.43	2.04
3	16.80	11.60	11.50	13.80	9.70	11.00	12.40	9.70	20.70	2.31	5.34
4	22.00	17.40	15.60	20.70	15.80	15.30	17.80	15.30	26.00	2.62	6.88
5	27.20	24.30	21.80	26.00	22.30	21.30	23.82	21.30	31.20	2.20	4.86
6	34.00	33.50	30.60	30.40	31.20	27.00	31.12	27.00	40.40	2.30	5.29
7	43.80	41.00	40.40	37.40	40.00	36.20	39.80	36.20	49.50	2.47	6.09
8	50.60	53.00	49.50	49.50	49.30	45.50	49.57	45.50	57.10	2.22	4.91
9	61.80	60.00	57.00	57.10	57.00	55.30	58.03	55.30	65.90	2.18	4.76
10	68.00	65.60	61.20	65.90	64.30	60.90	64.32	60.90	69.80	2.55	6.52
11	68.00	69.00	66.60	69.80	69.20	67.80	68.40	66.60	70.70	1.06	1.12
12	68.60	71.50	69.80	70.50	70.50	70.70	70.27	68.60	71.80	0.90	0.80
13	69.80	72.30	69.80	70.80	71.80	71.60	71.02	69.80	72.30	0.97	0.93
14	69.20	72.60	68.00	70.80	72.00	71.00	70.60	68.00	72.60	1.57	2.48
15	69.00	72.40	66.50	68.50	71.60	69.90	69.65	66.50	72.40	1.96	3.85
16	68.80	72.20	64.80	67.40	70.50	67.80	68.58	64.80	72.20	2.35	5.52
17	68.20	71.80	62.20	64.40	70.00	65.90	67.08	64.40	71.80	3.28	10.74
18	67.30	71.80	60.00	61.80	68.70	63.30	65.48	60.00	71.80	4.12	16.99
19	63.60	71.00	56.00	59.80	67.30	60.50	63.03	56.00	71.00	4.97	24.72
20	61.60	69.00	54.00	58.90	66.10	58.30	61.32	54.00	69.00	5.01	25.08
21	59.60	63.80	53.50	57.80	60.80	55.40	58.48	53.50	63.80	3.41	11.61
22	60.00	61.00	52.60	57.30	59.80	54.40	57.52	52.60	61.00	3.09	9.57
23	60.00	59.00	51.00	56.50	59.60	51.70	56.30	51.00	60.00	3.68	13.53
24	59.00	59.30	50.00	55.80	57.90	50.40	55.40	50.00	59.30	3.85	14.79
25		57.80		53.50	58.30	50.00	54.90	53.50	58.30	3.39	11.49
26					57.80			57.80	57.80		

TABLE XIX
STATISTICAL ANALYSIS
UNINOCULATED 100-FOLD CONC.

UNINOCULATED TRIALS
MEDIA CONC. 100X
FUEL CELL OUTPUT, millivolts

TRIAL / TIME,HRS	25	26	27	28	29	30	AVERAGE	MINIMUM	MAXIMUM	STD DEV	VARIANCE
1	3.60	3.00	2.40	3.10	1.10	3.20	2.73	1.10	3.60	0.81	0.66
2	6.00	6.00	4.30	5.60	3.80	4.30	5.00	3.80	6.00	0.89	0.80
3	8.00	8.00	9.00	7.40	6.30	6.80	7.58	6.30	9.00	0.88	0.77
4	10.60	10.60	14.50	9.50	9.10	12.20	11.08	9.10	14.50	1.82	3.30
5	15.00	13.50	18.40	12.20	11.70	17.50	14.72	11.70	18.40	2.53	6.38
6	17.00	15.00	20.30	13.90	13.90	21.30	16.90	13.90	21.30	2.96	8.76
7	18.20	18.20	21.00	15.60	17.00	22.40	18.73	15.60	22.40	2.31	5.33
8	19.80	20.50	21.50	18.60	19.10	21.80	20.22	18.60	21.80	1.17	1.38
9	21.00	21.80	22.00	19.80	20.40	22.50	21.25	19.80	22.50	0.94	0.89
10	22.60	23.00	22.60	22.00	21.10	23.40	22.45	21.10	23.40	0.74	0.55
11	23.00	23.40	22.80	22.40	22.20	22.40	22.70	22.20	23.40	0.41	0.17
12	23.20	23.00	22.50	22.10	22.30	22.30	22.57	22.10	23.20	0.40	0.16
13	23.60	22.00	22.20	22.70	22.10	22.60	22.53	22.00	23.60	0.54	0.29
14	23.60	19.80	22.00	23.30	20.00	22.70	21.90	19.80	23.60	1.50	2.25
15	23.60	18.00	22.00	23.20	18.20	20.60	20.93	18.00	23.60	2.22	4.93
16	23.20	16.30	20.00	22.80	16.60	18.50	19.57	16.30	23.20	2.72	7.41
17	21.40	13.50	17.00	19.50	13.80	16.30	16.92	13.50	21.40	2.84	8.09
18	18.50	12.30	14.00	18.00	12.60	12.30	14.62	12.30	18.50	2.64	6.95
19	15.50	11.60	11.80	16.90	13.00	8.00	12.80	8.00	16.90	2.88	8.27
20	13.40	10.80	11.60	15.00	12.70	9.10	12.10	9.10	15.00	1.89	3.57
21	12.00	10.50	11.00	14.00	13.00	6.10	11.10	6.10	14.00	2.52	6.37
22	11.20	11.00	10.00	12.30	12.50	6.20	10.53	6.20	12.50	2.11	4.45
23	11.00	10.20	11.00	12.10	10.90	5.90	10.18	5.90	12.10	1.99	3.98
24	10.50	11.00	10.00	11.80		5.10	9.68	5.10	11.00	2.37	5.60
25											
26											

TABLE XX
STATISTICAL ANALYSIS
INOCULATED 100-FOLD CONC.

INOCULATED TRIALS
MEDIA CONC. 100X
FUEL CELL OUTPUT, millivolts

TIME,HRS \ TRIAL	31	32	33	34	35	36	AVERAGE	MINIMUM	MAXIMUM	STD DEV	VARIANCE
1	5.30	6.30	6.00	4.10	5.00	4.70	5.23	4.10	6.30	0.75	0.56
2	10.20	12.00	10.30	8.80	10.30	8.20	9.97	8.20	12.00	1.22	1.48
3	15.40	16.00	16.00	16.30	14.80	13.60	15.35	13.60	16.30	0.92	0.85
4	21.40	21.60	23.50	23.60	20.00	21.00	21.85	21.00	23.60	1.30	1.70
5	28.50	29.50	31.60	30.30	28.30	28.30	29.42	28.30	31.60	1.22	1.48
6	37.30	34.50	38.00	39.20	34.20	39.10	37.05	34.20	39.20	2.02	4.07
7	47.80	44.70	46.70	48.70	41.00	48.50	46.23	41.00	48.70	2.70	7.27
8	59.00	54.00	56.00	60.60	50.70	56.70	56.17	50.70	60.60	3.23	10.43
9	66.50	61.30	63.50	69.50	62.50	65.30	64.77	61.30	69.50	2.72	7.41
10	74.10	70.20	70.20	76.80	71.60	72.80	72.62	70.20	76.80	2.33	5.41
11	79.80	76.70	79.30	82.10	75.50	79.30	78.78	75.50	82.10	2.15	4.61
12	81.50	79.80	80.60	83.50	80.60	82.80	81.47	79.80	83.50	1.30	1.70
13	79.60	80.90	80.70	83.40	81.40	83.70	81.62	79.60	83.70	1.47	2.16
14	79.30	81.20	80.80	84.30	82.50	84.20	82.05	79.30	84.30	1.81	3.29
15	80.00	80.80	80.70	84.50	83.00	84.00	82.17	80.00	84.50	1.74	3.04
16	79.60	80.50	80.30	82.60	79.90	83.70	81.10	79.60	83.70	1.51	2.28
17	75.80	79.90	79.70	80.30	77.80	82.60	79.35	75.80	82.60	2.12	4.48
18	70.30	73.50	79.00	75.30	75.70	81.60	75.90	70.30	81.60	3.64	13.27
19	64.30	67.50	77.30	66.90	72.00	80.30	71.38	64.30	80.30	5.78	33.37
20	60.90	62.30	70.10	64.30	66.30	75.60	66.58	60.90	75.60	4.99	24.93
21	60.10	60.40	64.00	61.90	63.40	69.80	63.27	60.10	69.80	3.25	10.56
22	59.30	60.50	59.70	60.60	61.30	64.50	61.00	59.30	64.50	1.70	2.87
23	60.70	60.70	58.90	60.20	59.30	61.80	60.27	58.90	61.80	0.96	0.92
24	60.50	59.30	57.30	59.50	57.00	59.70	58.97	57.00	60.50	1.32	1.75
25	59.30			59.00			59.15	59.00	59.30	0.15	0.02
26				57.70				57.70	57.70		

TABLE XXI
STATISTICAL ANALYSIS
UNINOCULATED 1000-FOLD CONC.

UNINOCULATED TRIALS
MEDIA CONC. 1000X
FUEL CELL OUTPUT, millivolts

TRIAL / TIME,HRS	37	38	39	40	41	42	AVERAGE	MINIMUM	MAXIMUM	STD DEV	VARIANCE
1	3.60	4.80	2.30	2.60	3.60	3.70	3.43	2.30	4.80	0.81	0.66
2	5.80	6.60	4.00	5.70	6.40	6.10	5.77	4.00	6.60	0.85	0.72
3	9.10	10.00	6.50	8.40	8.50	7.70	8.37	6.50	10.00	1.09	1.19
4	13.60	12.60	8.40	12.60	11.50	9.50	11.37	8.40	13.60	1.84	3.39
5	15.80	15.00	11.80	16.10	14.30	12.60	14.27	11.80	16.10	1.59	2.52
6	16.70	18.40	15.30	17.90	18.20	15.70	17.03	15.30	18.40	1.22	1.48
7	18.80	21.30	17.90	19.20	20.80	19.50	19.58	17.90	21.30	1.16	1.34
8	21.20	23.10	21.00	21.30	21.80	20.30	21.45	21.00	23.10	0.86	0.74
9	22.40	24.40	23.80	23.40	22.60	22.40	23.17	22.40	24.40	0.76	0.58
10	24.50	24.60	23.80	25.20	24.40	25.00	24.58	23.80	25.20	0.45	0.20
11	24.60	24.40	23.60	25.60	25.80	24.50	24.75	23.60	25.80	0.75	0.56
12	24.20	24.40	23.50	24.50	27.00	25.00	24.77	23.50	27.00	1.09	1.20
13	24.50	24.30	22.40	23.80	26.30	23.00	24.05	22.40	26.30	1.24	1.54
14	24.40	24.00	21.80	23.60	25.60	22.20	23.60	21.80	25.60	1.29	1.67
15	24.00	23.80	20.50	22.20	25.30	21.30	22.85	20.50	25.30	1.66	2.76
16	23.00	23.30	19.00	22.00	24.00	20.80	22.02	19.00	24.00	1.69	2.85
17	20.60	21.70	18.00	21.10	21.70	18.30	20.23	18.00	21.70	1.52	2.32
18	17.50	16.50	15.30	18.00	18.60	14.70	16.77	14.70	18.60	1.41	1.99
19	13.60	12.80	13.30	14.50	15.70	12.20	13.68	12.20	15.70	1.15	1.31
20	11.80	11.80	9.50	10.80	14.50	11.60	11.67	9.50	14.50	1.50	2.25
21	11.30	10.00	8.40	10.70	13.90	10.20	10.75	8.40	13.90	1.66	2.77
22	10.20	10.30	8.20	10.60	12.70	9.30	10.22	8.20	12.70	1.37	1.87
23	10.00	10.20	9.10	8.40	11.70	8.50	9.65	8.50	11.70	1.14	1.30
24	10.00	10.40	9.50		8.30		9.55	8.30	10.40	0.79	0.62
25			9.80		7.70		8.75	7.70	9.80	1.05	1.10
26											

TABLE XXII
STATISTICAL ANALYSIS
INOCULATED 1000-FOLD CONC.

INOCULATED TRIALS
MEDIA CONC. 1000X
FUEL CELL OUTPUT, millivolts

TRIAL / TIME,HRS	43	44	45	46	47	48	AVERAGE	MINIMUM	MAXIMUM	STAN.DEV	VARIANCE
1	6.10	3.90	3.00	4.60	3.80	3.80	4.20	3.00	6.10	0.97	0.94
2	9.10	9.30	7.40	8.30	8.20	7.30	8.27	7.30	9.30	0.76	0.58
3	15.40	15.00	12.60	14.50	13.50	12.80	13.97	12.60	15.40	1.07	1.14
4	19.30	22.00	17.30	20.70	19.10	18.00	19.40	17.30	22.00	1.58	2.49
5	27.20	32.90	20.20	28.40	28.30	24.50	26.92	20.20	32.90	3.89	15.16
6	35.00	40.00	30.30	35.90	38.40	34.30	35.65	30.30	40.00	3.09	9.57
7	43.40	54.70	40.40	41.70	54.90	44.00	46.52	40.40	54.90	5.97	35.65
8	53.00	67.30	49.50	49.80	67.10	53.60	56.72	49.50	67.30	7.56	57.21
9	63.30	77.50	61.40	61.90	68.50	63.40	66.00	61.40	77.50	5.64	31.75
10	72.50	86.00	74.70	74.00	87.00	73.50	77.95	72.50	87.00	6.09	37.06
11	82.00	87.80	83.40	85.10	90.40	81.50	85.03	81.50	90.40	3.18	10.14
12	87.40	87.80	87.00	89.60	88.20	87.00	87.83	87.00	89.60	0.90	0.81
13	88.30	87.90	87.60	91.00	88.40	88.50	88.62	87.60	91.00	1.11	1.23
14	88.40	87.50	86.50	87.70	90.50	89.00	88.27	86.50	90.50	1.26	1.60
15	87.10	87.80	87.40	87.70	91.50	90.00	88.58	87.10	91.50	1.61	2.58
16	86.50	86.80	87.30	89.00	89.00	90.30	88.15	86.50	90.30	1.37	1.89
17	85.00	78.70	86.70	86.20	82.30	89.80	84.78	78.70	89.80	3.51	12.34
18	81.70	71.80	86.90	81.50	71.40	89.70	80.50	71.80	89.70	6.91	47.76
19	78.00	69.80	84.50	76.40	68.50	86.40	77.27	69.80	86.40	6.70	44.94
20	72.70	67.00	79.20	70.70	68.70	82.10	73.40	67.00	82.10	5.48	30.03
21	67.30	67.00	75.30	66.30	69.00	76.50	70.23	66.30	76.50	4.10	16.83
22	65.00	65.60	73.00	65.80	67.30	73.00	68.28	65.00	73.00	3.41	11.60
23	64.00	65.70	71.40	65.00	66.30	72.90	67.55	64.00	72.90	3.35	11.26
24	62.60	64.00	69.90	63.40	65.00	71.80	66.12	62.60	71.80	3.47	12.01
25			68.70	62.50		70.40	67.20	62.50	70.40	3.40	11.53
26			67.80			68.50	68.15	67.80	68.50	0.35	0.12
27						66.90	66.90	66.90	66.90		
28						65.80	65.80		65.80		

TABLE XXIII
STATISTICAL ANALYSIS
ACTIVATED SLUDGE FUEL CELL

ACTIVATED SLUDGE TRIALS
MEDIA CONC. 1X
FUEL CELL OUTPUT, millivolts

TRIAL / TIME,HRS	1	2	3	4	5	6	AVERAGE	MINIMUM	MAXIMUM	STD DEV	VARIANCE
1	3.50	1.90	5.40	3.10	3.40	3.30	3.43	1.90	5.40	1.03	1.06
2	6.00	4.40	10.00	8.00	4.50	8.00	6.82	4.40	10.00	2.03	4.13
3	9.60	7.80	13.40	12.80	5.60	11.90	10.18	5.60	13.40	2.80	7.86
4	12.40	10.30	14.30	13.90	6.60	12.20	11.62	6.60	14.30	2.59	6.71
5	15.80	14.20	15.70	14.50	9.30	10.20	13.28	9.30	15.80	2.58	6.64
6	22.00	22.10	22.80	19.00	12.70	14.00	18.77	12.70	22.80	4.03	16.24
7	28.00	27.30	26.00	20.90	15.00	18.20	22.57	15.00	28.00	4.88	23.80
8	33.10	31.80	30.30	26.20	22.40	22.70	27.75	22.40	33.10	4.24	18.01
9	36.70	36.10	35.80	30.60	29.90	25.80	32.48	25.80	36.70	4.02	16.12
10	38.40	37.30	38.20	33.90	31.50	27.30	34.43	27.30	38.40	4.04	16.32
11	38.10	36.40	38.00	37.30	30.40	29.10	34.88	29.10	38.10	3.69	13.62
12	37.40	36.50	39.90	38.20	34.10	34.90	36.83	34.10	39.90	1.95	3.82
13	38.00	35.50	38.70	37.90	35.10	33.40	36.43	33.40	38.70	1.90	3.60
14	37.80	35.00	39.30	37.10	35.80	32.40	36.23	32.40	39.30	2.20	4.84
15	39.50	36.20	40.00	36.20	37.70	34.00	37.27	34.00	40.00	2.07	4.27
16	36.30	36.80	39.00	37.10	37.00	37.70	37.32	36.30	39.00	0.86	0.74
17	35.40	37.00	40.20	36.80	38.40	37.80	37.60	35.40	40.20	1.49	2.21
18	38.20	36.00	39.10	37.70	39.80	38.60	38.23	36.00	39.80	1.20	1.44
19	36.00	36.10	39.00	37.00	40.00	38.20	37.72	36.00	40.00	1.48	2.19
20	37.30	36.10	37.80	37.00	40.30	36.60	37.52	36.10	40.30	1.35	1.83
21	36.00	35.90	37.10	38.50	39.80	38.20	37.58	35.90	39.80	1.40	1.95
22	36.40	36.20	36.20	39.80	38.60	39.80	37.83	36.20	39.80	1.62	2.62
23	36.00	36.10	36.80	39.70	37.60	38.40	37.43	36.00	39.70	1.31	1.72
24	34.90	34.10	36.70	40.00	40.00	36.60	37.05	34.10	40.00	2.28	5.18
25	34.50			38.40	36.40	37.70	36.75	34.50	38.40	1.48	1.26
26				36.90	36.10		36.50	36.10	36.90	0.40	0.16

APPENDIX 4

LIST OF FIGURES

FIGURE I
FUEL CELL DESIGN

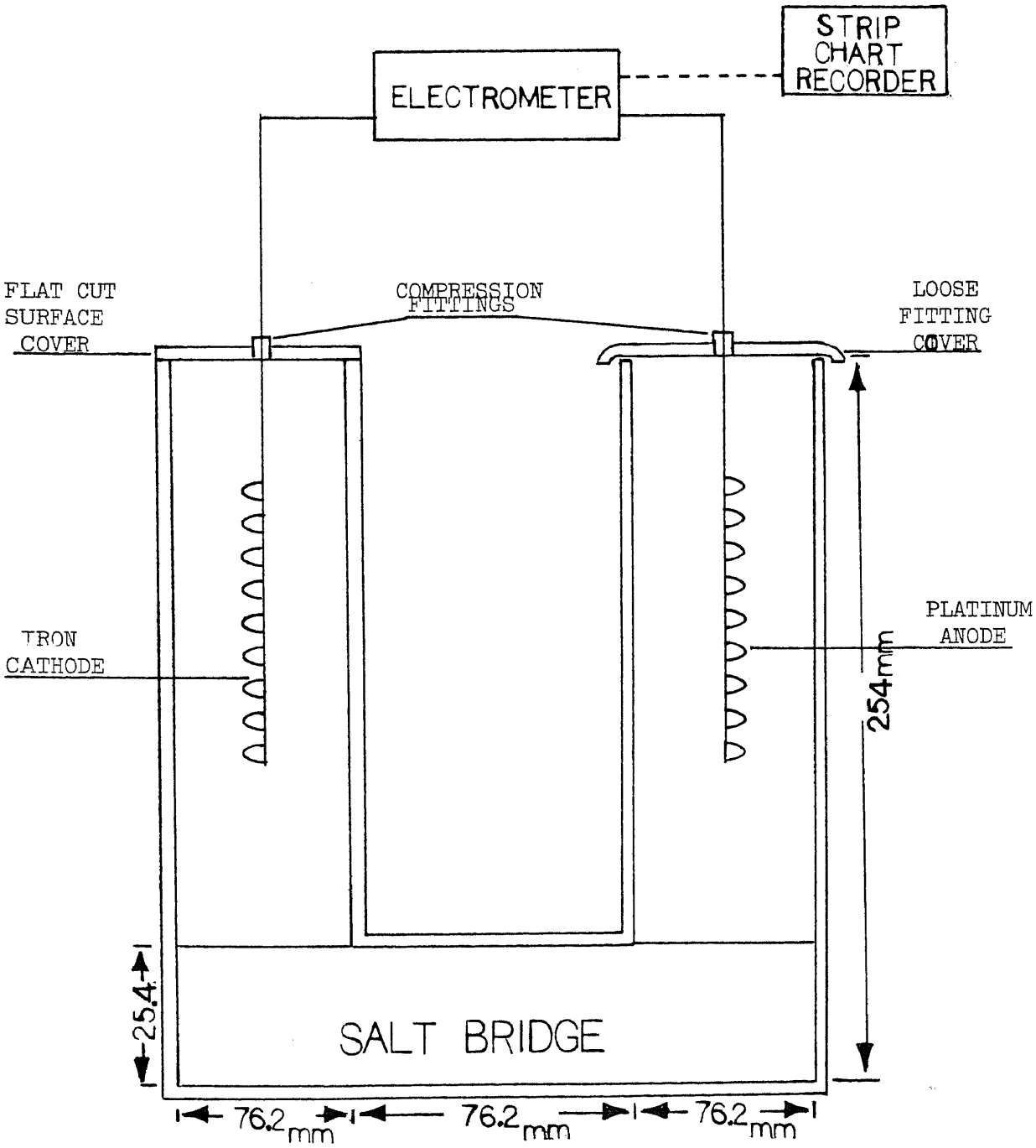


FIGURE II
FUEL CELL DESIGN

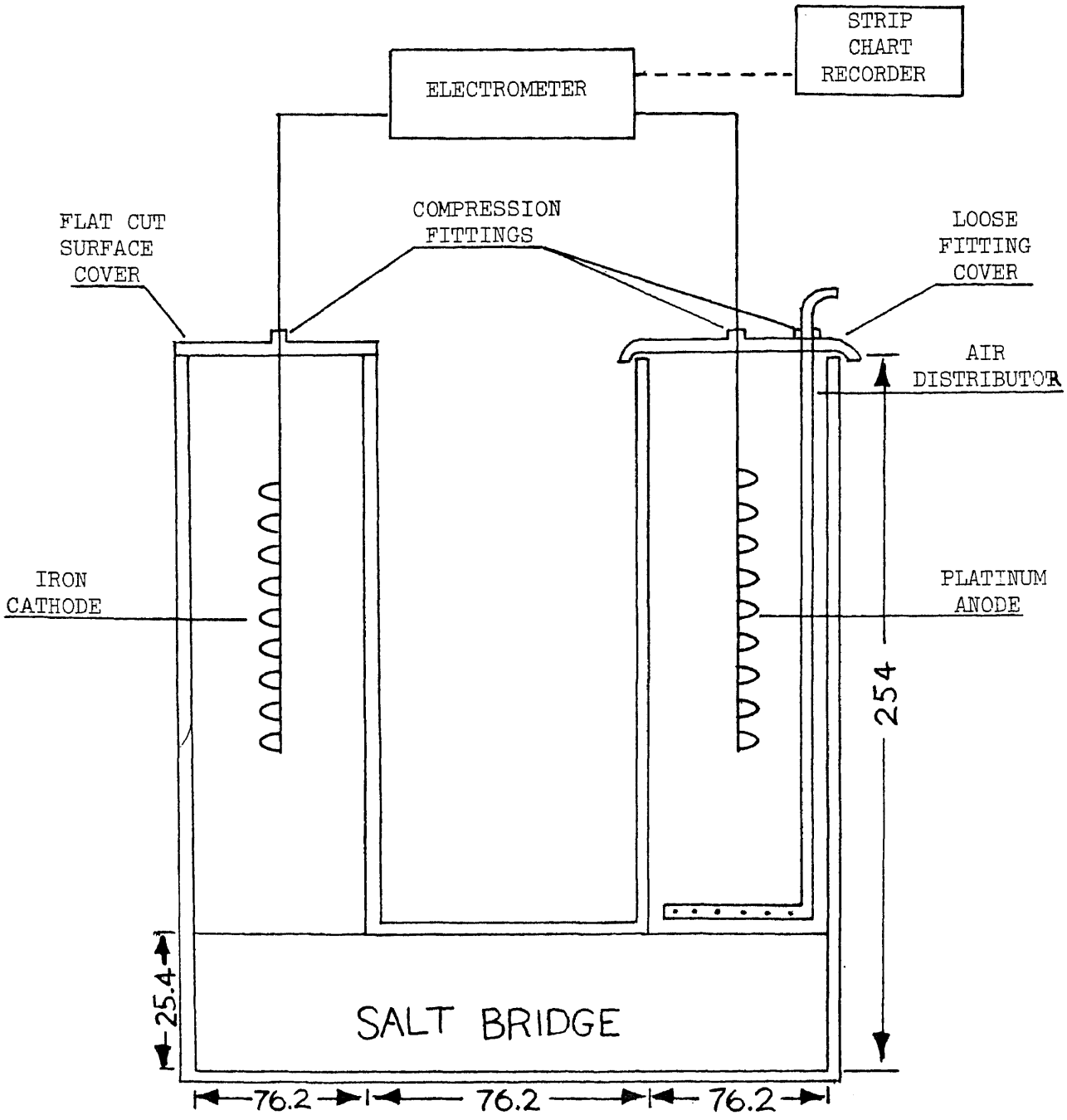
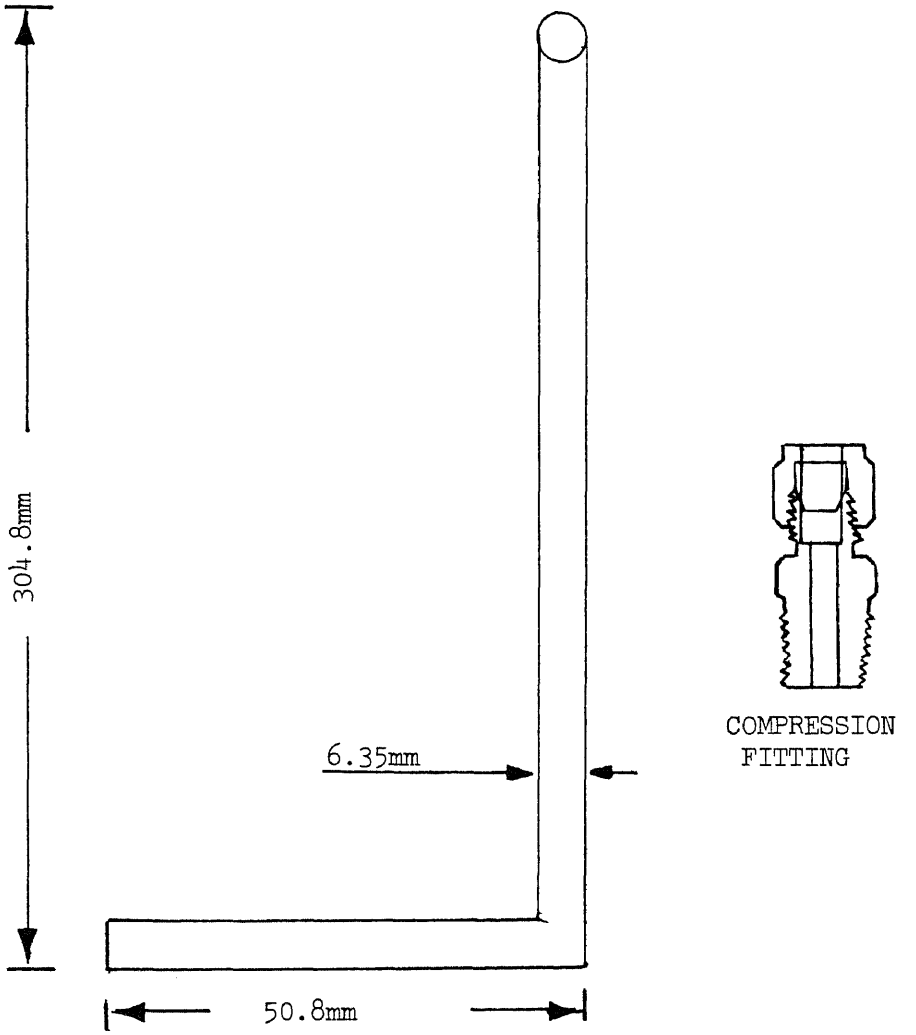
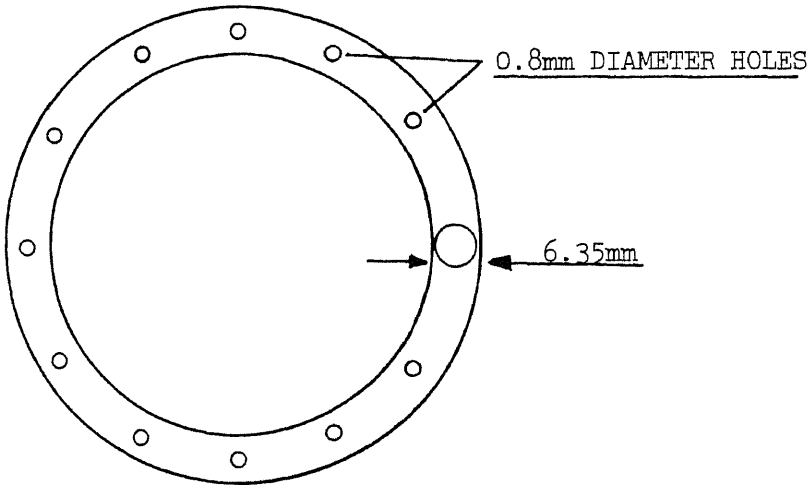


FIGURE III
FUEL CELL
CONSTRUCTION DETAILS





DISTRIBUTOR
SIDE AND PLAN VIEWS

FIGURE IV

FUEL CELL OUTPUT
vs
10 FOLD MEDIA CONCENTRATION

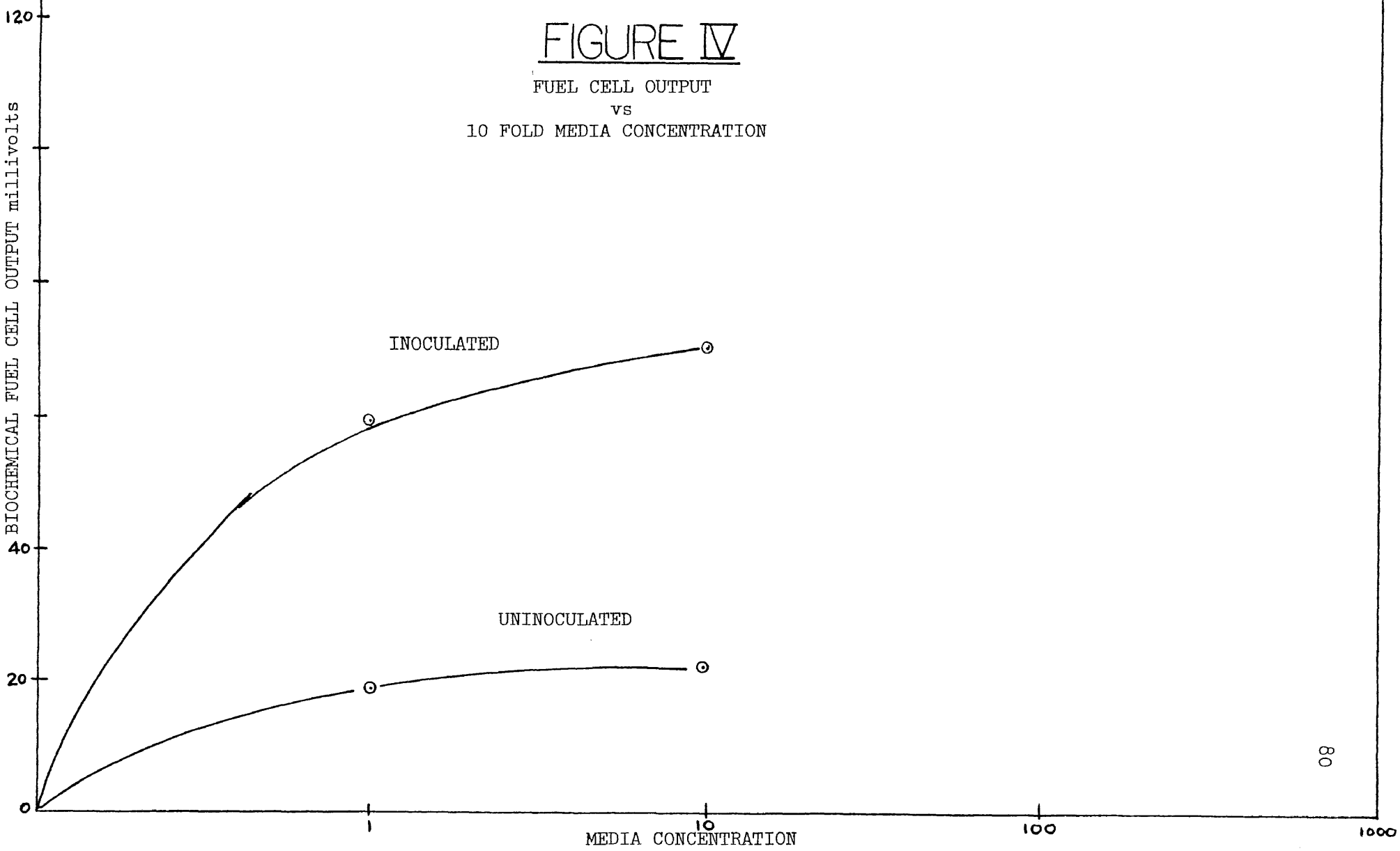
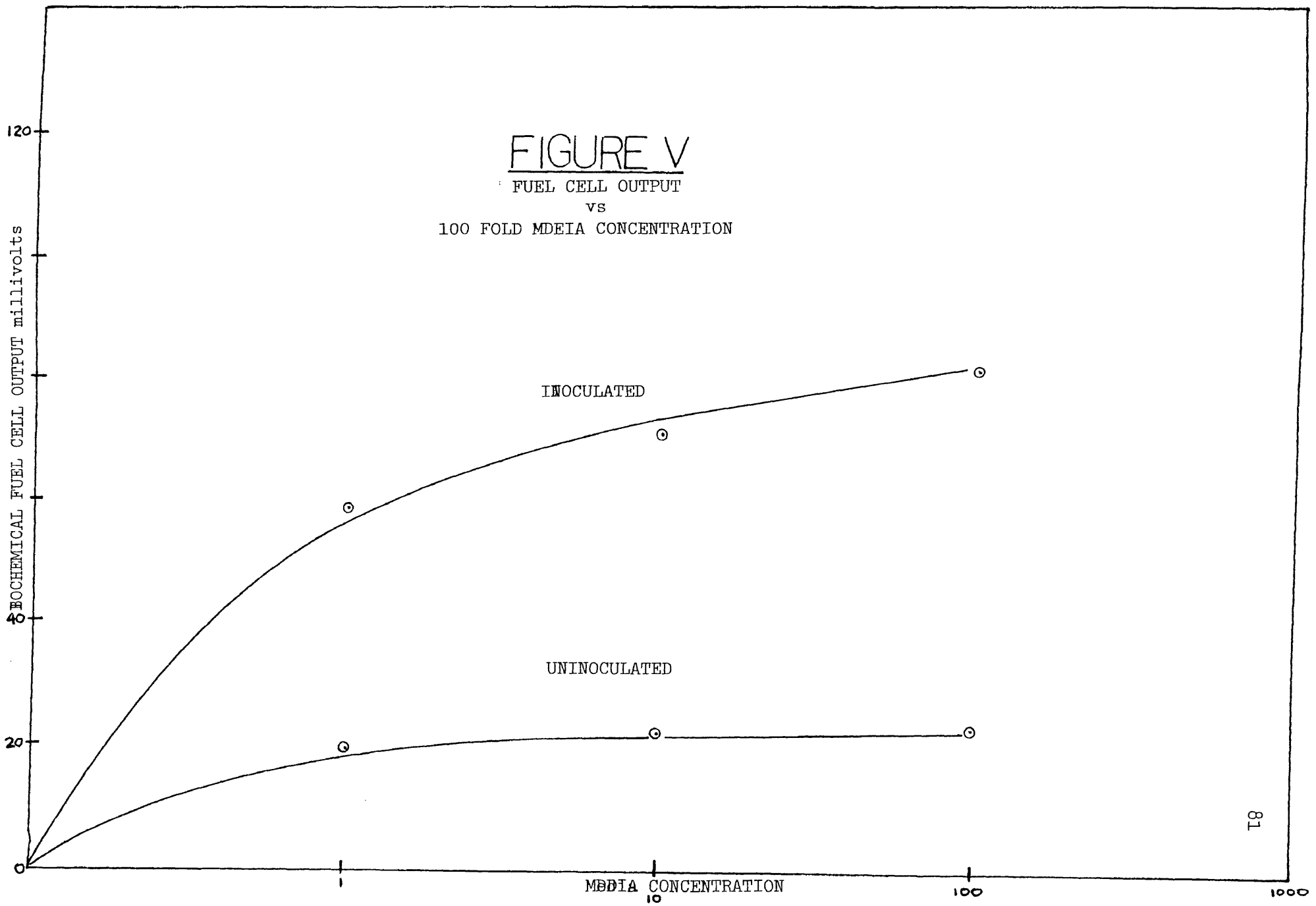


FIGURE V

FUEL CELL OUTPUT
vs
100 FOLD MDEIA CONCENTRATION



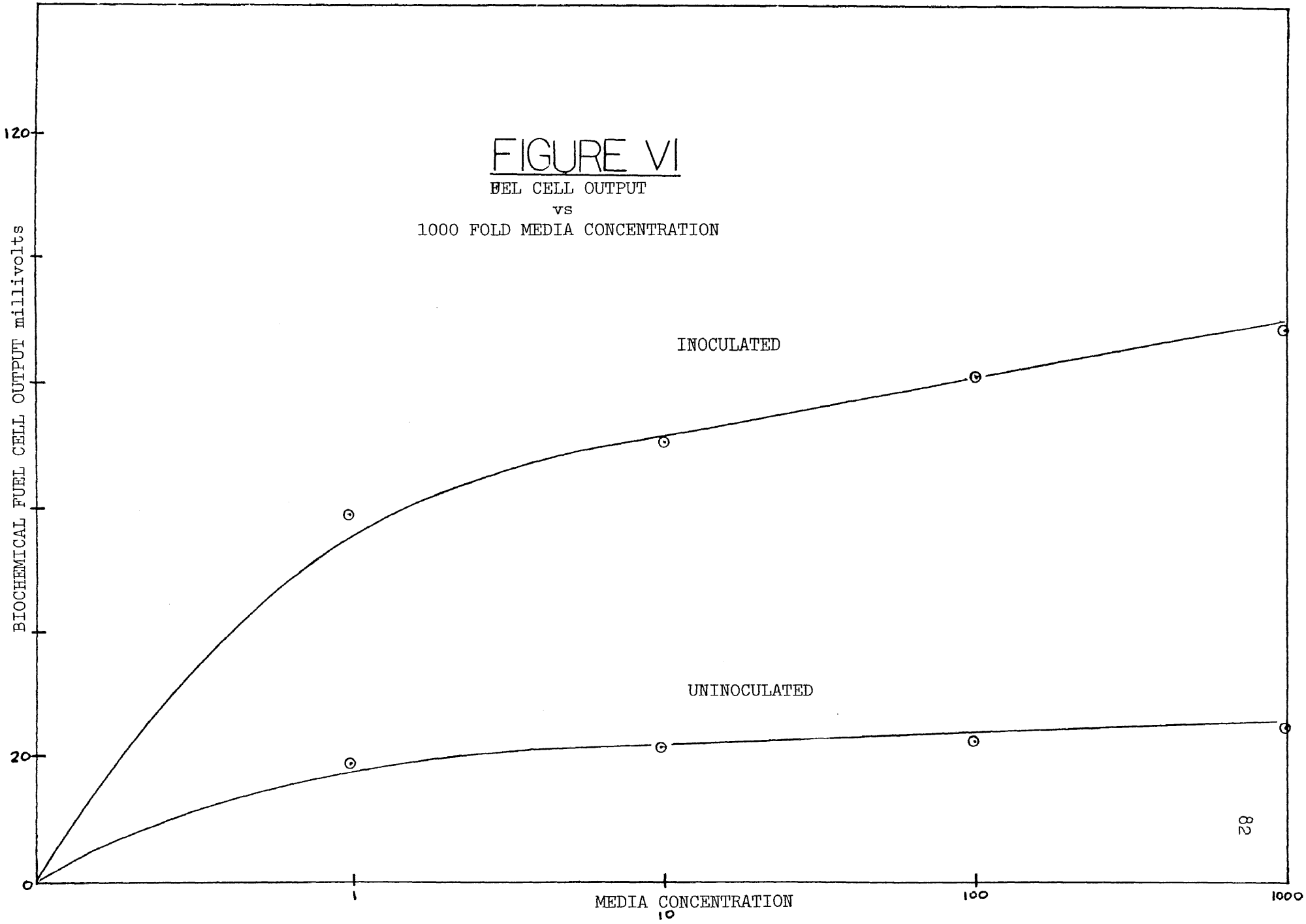
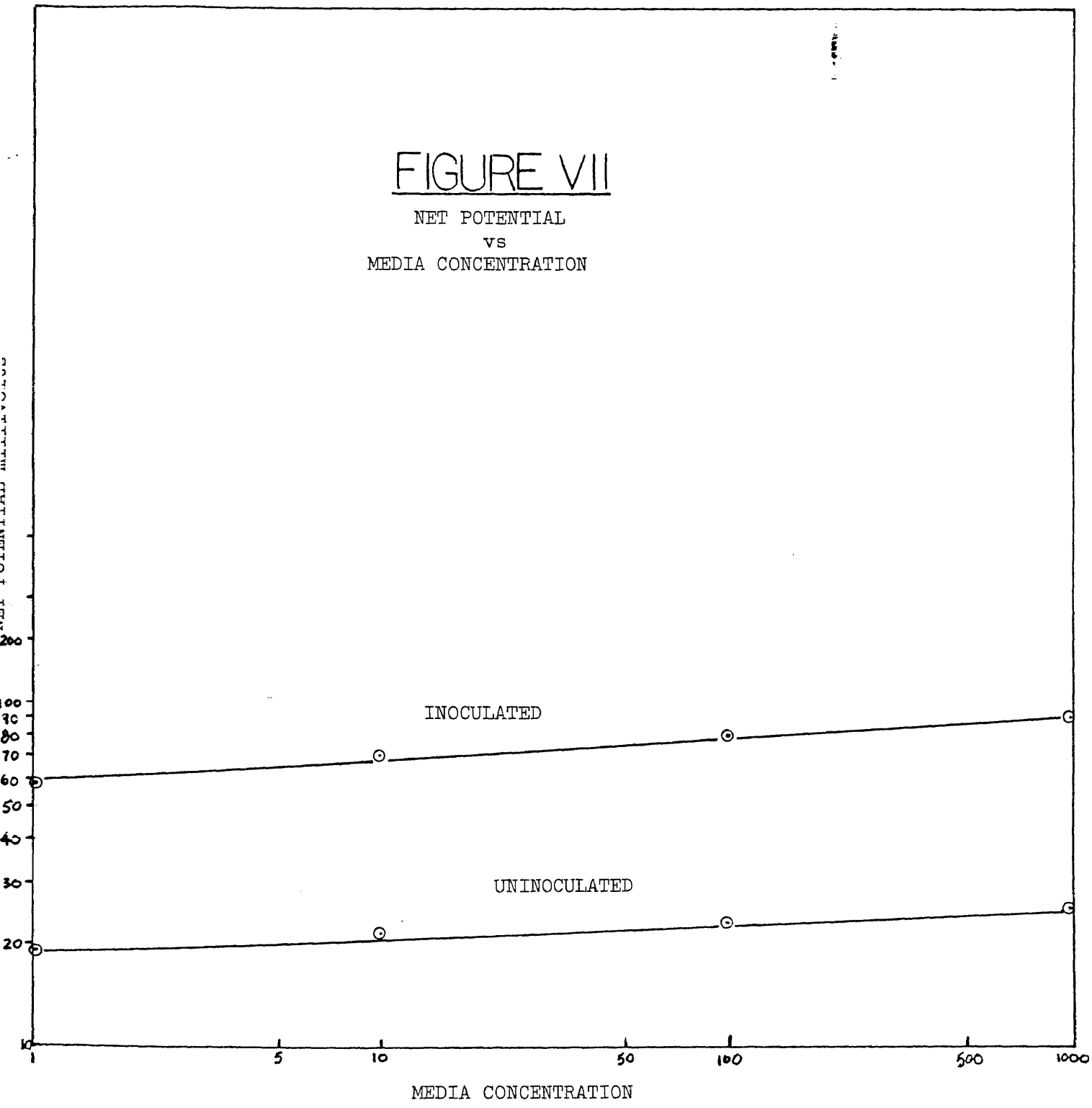


FIGURE VI
FUEL CELL OUTPUT
vs
1000 FOLD MEDIA CONCENTRATION
BIOCHEMICAL FUEL CELL OUTPUT millivolts
120
20
0
INOCULATED
UNINOCULATED
1
10
100
1000
MEDIA CONCENTRATION

FIGURE VII

NET POTENTIAL
vs
MEDIA CONCENTRATION



APPENDIX 5

LIST OF CHARTS

Chart 1
UNINOCULATED FUEL CELL OUTPUT
INITIAL CONC.

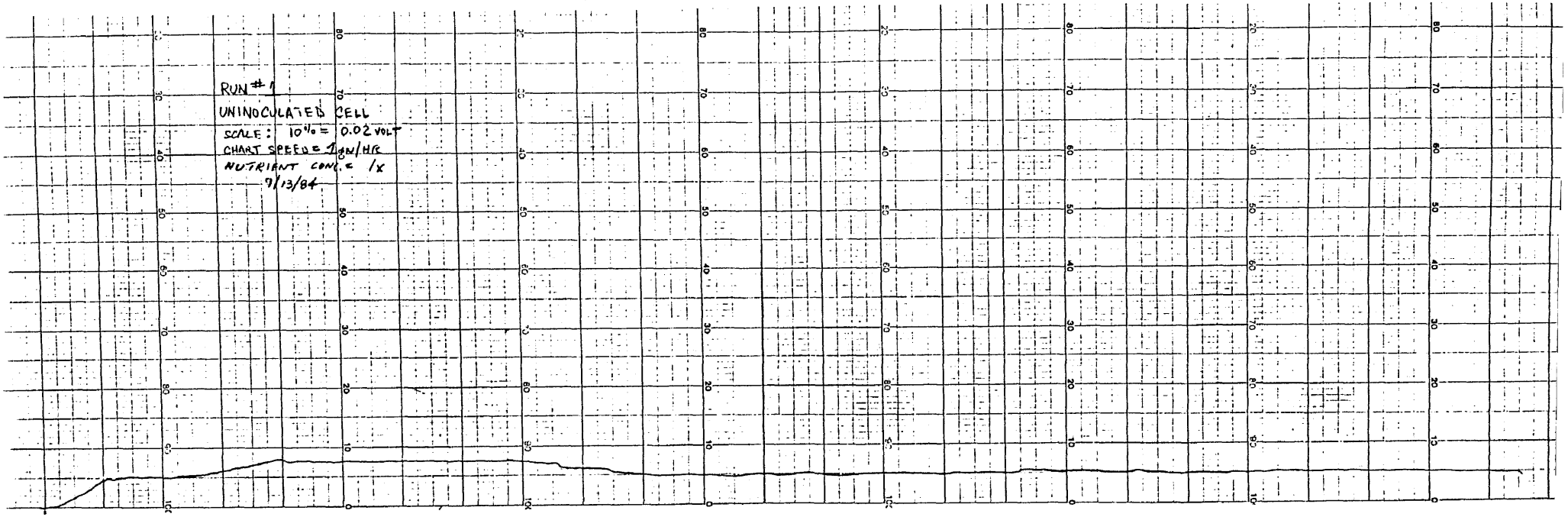


CHART 2
UNINOCULATED FEUL CELL OUTPUT
INITIAL CONC.

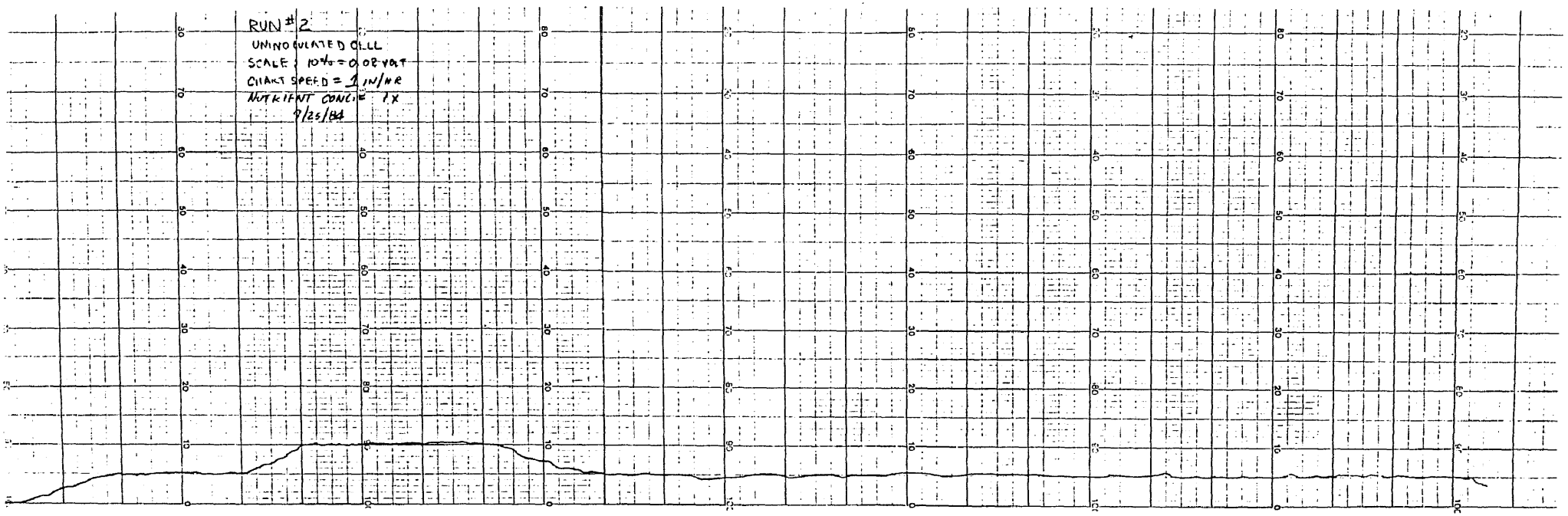


CHART 3
UNINOCULATED FUEL CELL OUTPUT
INITIAL CONC.

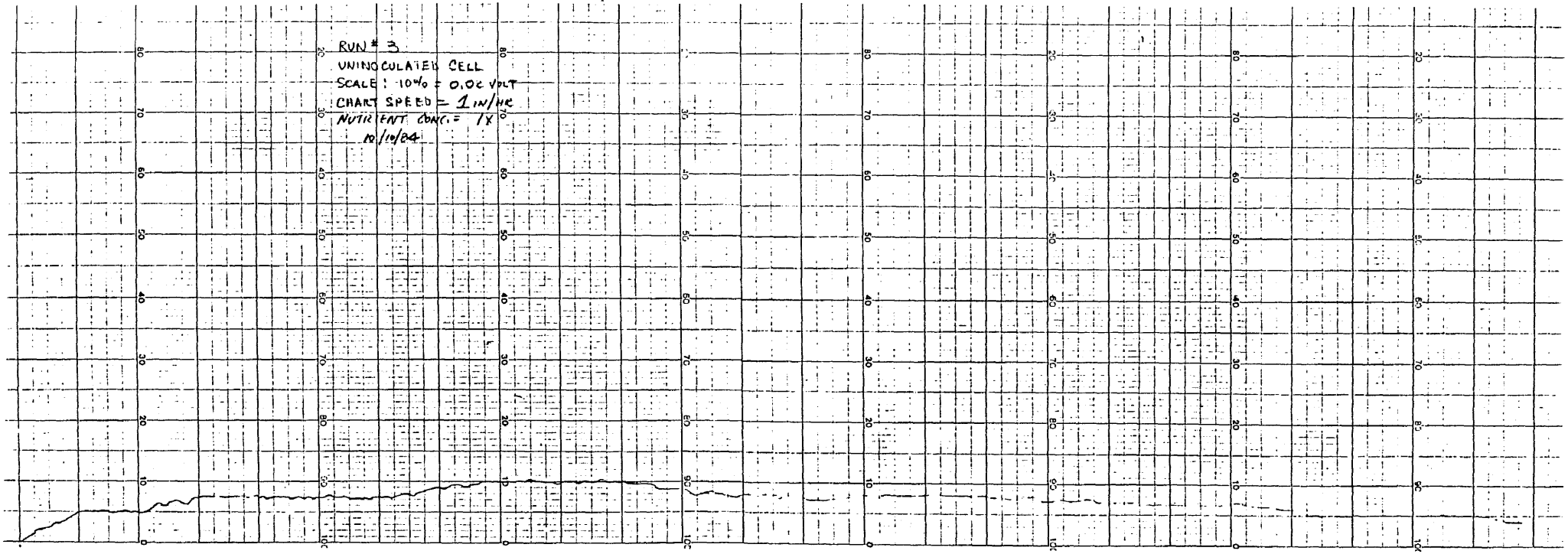


CHART 4
UNINOCULATED FUEL CELL OUTPUT
INITIAL CONC.

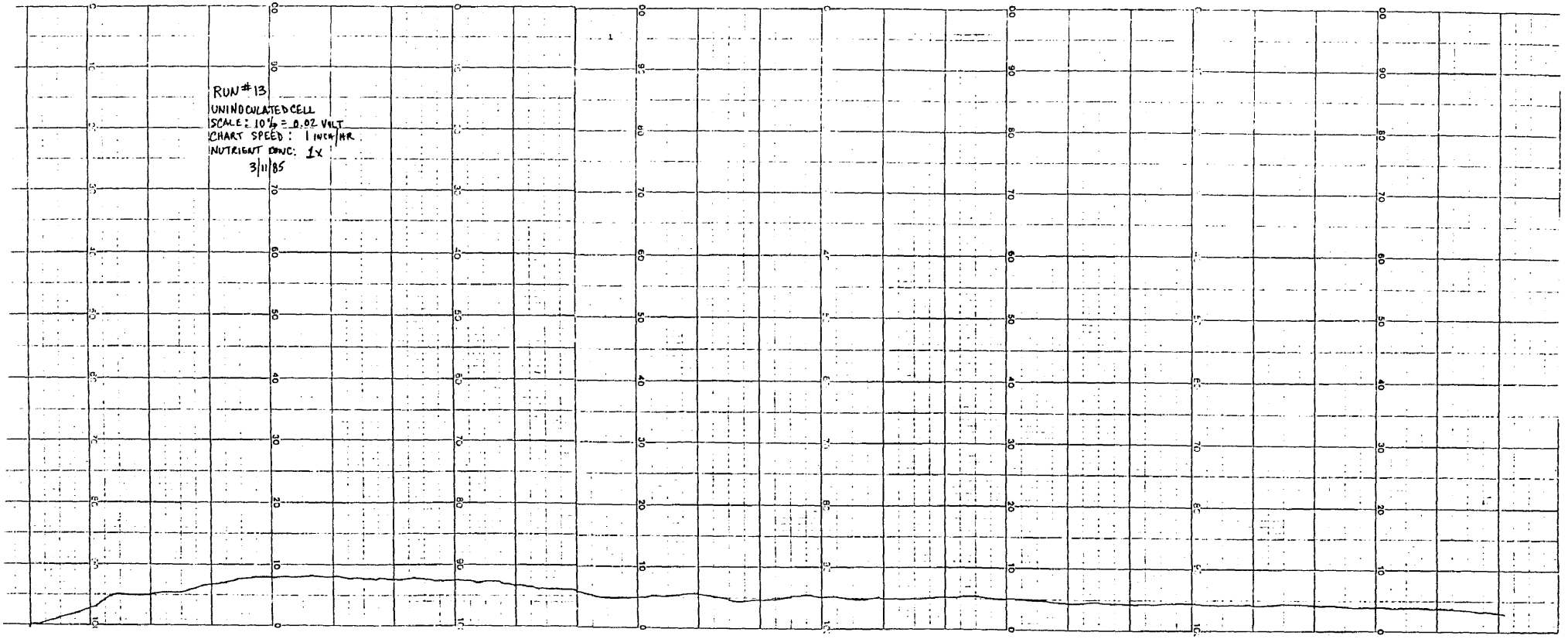


CHART 5
UNINOCULATED FUEL CELL OUTPUT
INITIAL CONC.

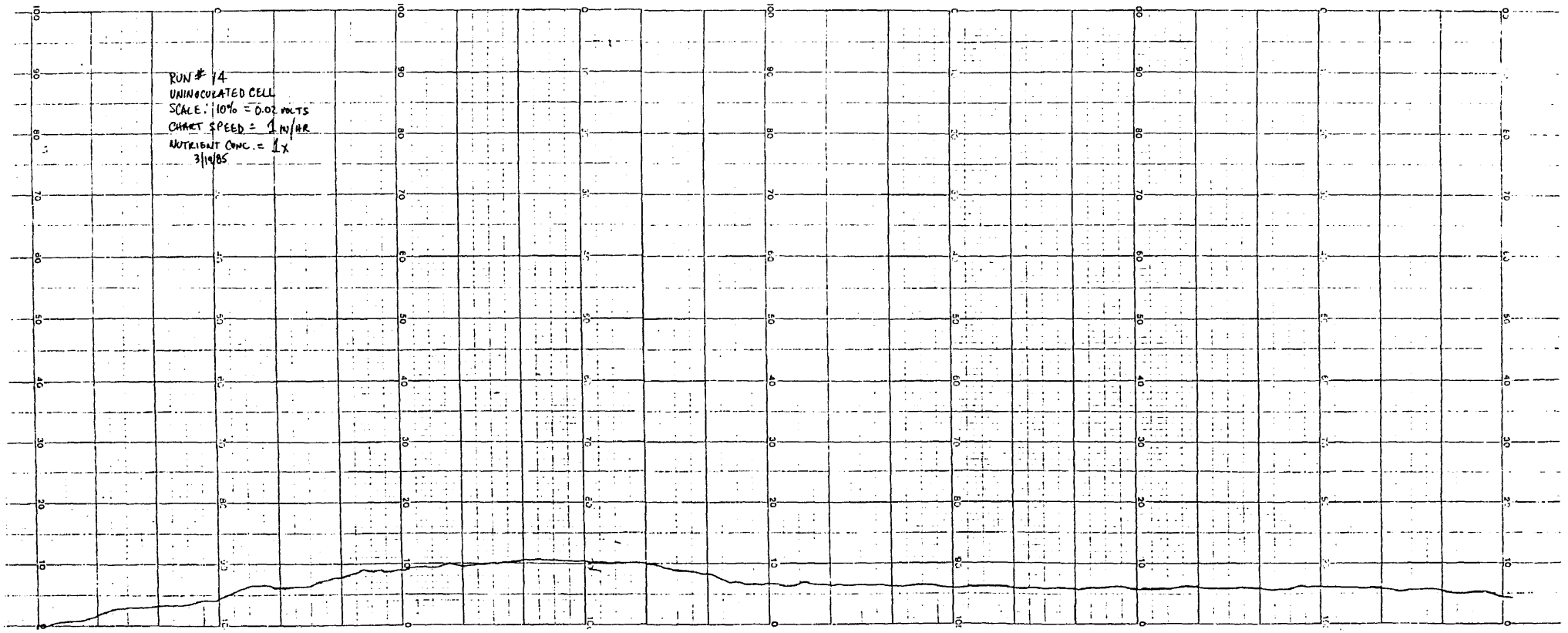


CHART 6
UNINOCULATED FUEL CELL OUTPUT
INITIAL CONC.

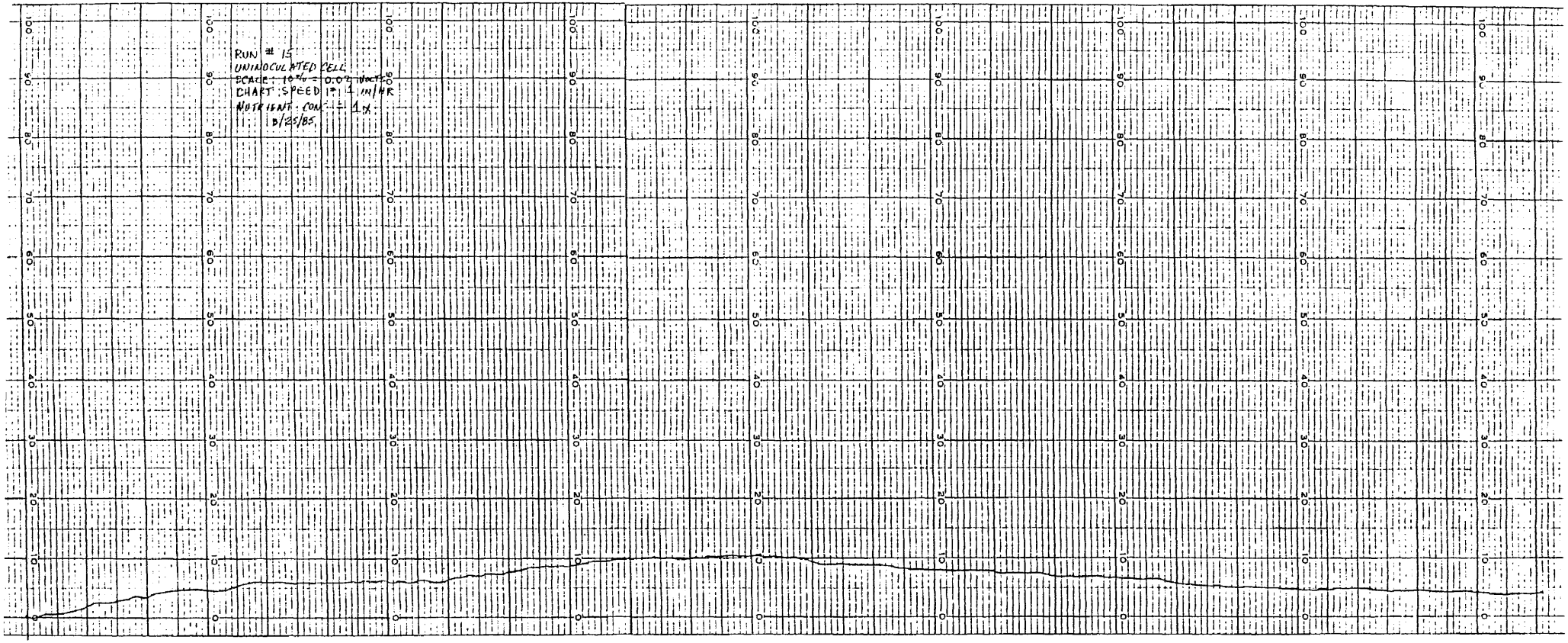


CHART 7
INOCULATED FUEL CELL OUTPUT
INITIAL CONC.

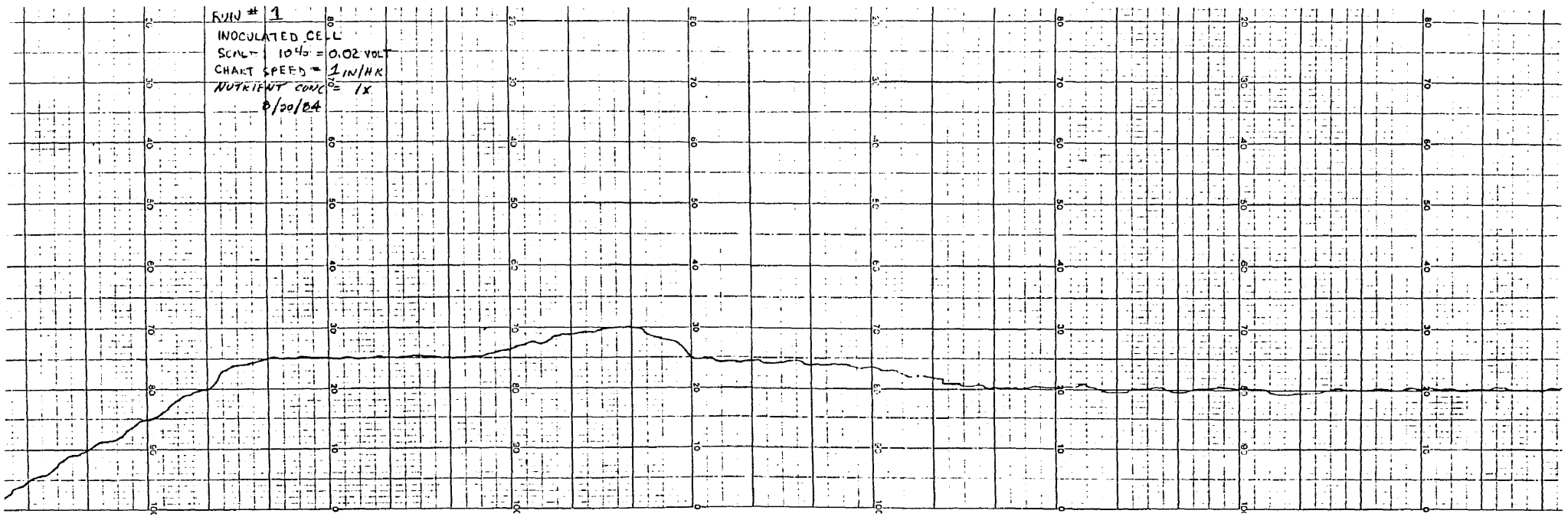


CHART 8
INOCULATED FUEL CELL OUTPUT
INITIAL CONC.

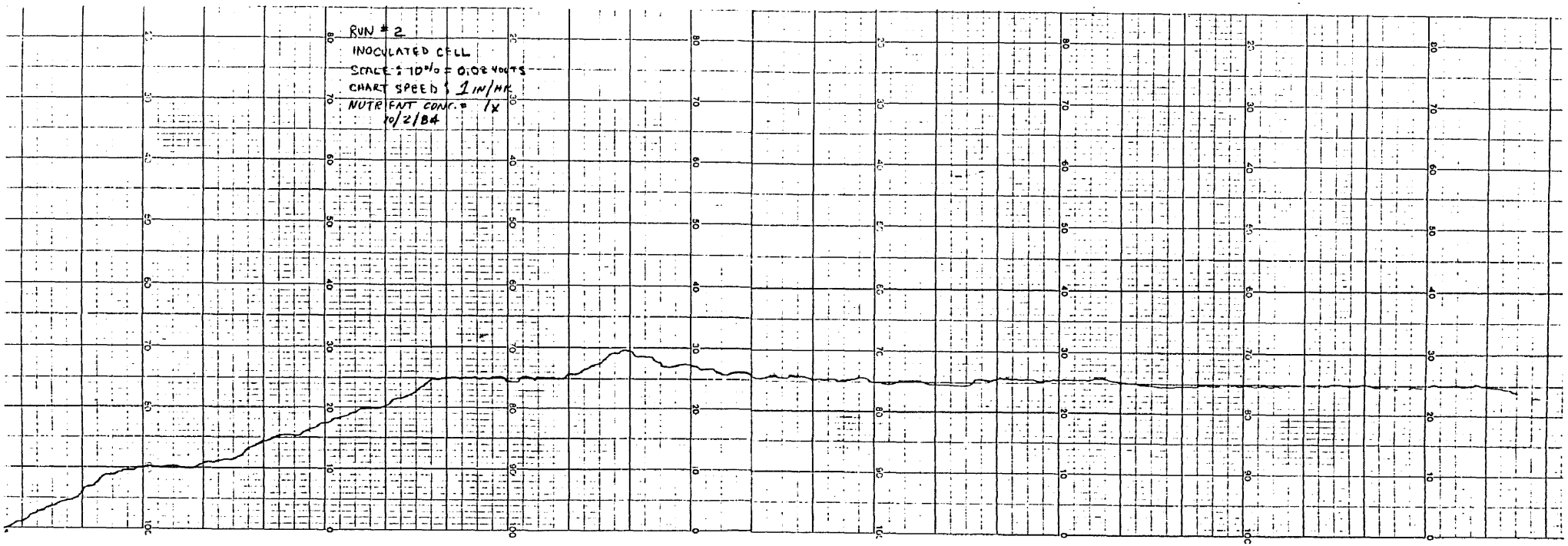


CHART 9
INOCULATED FUEL CELL OUTPUT
INITIAL CONC.

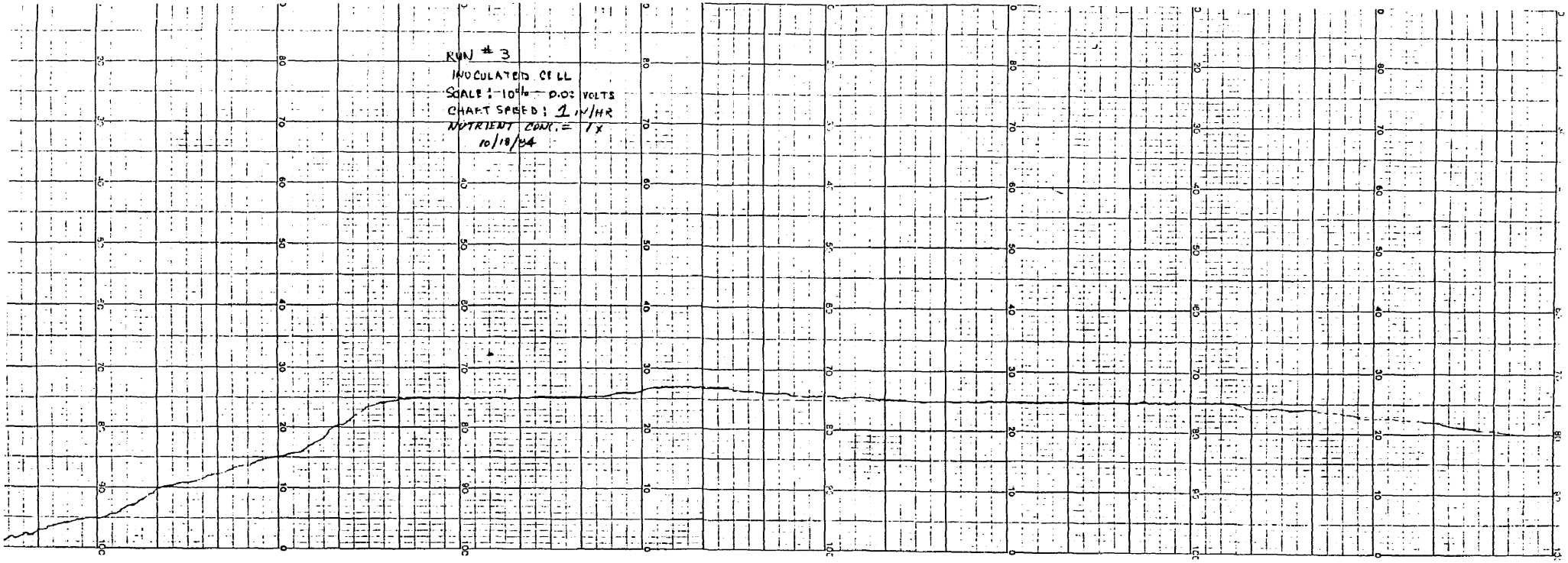


CHART 10
INOCULATED FUEL CELL OUTPUT
INITIAL CONC.

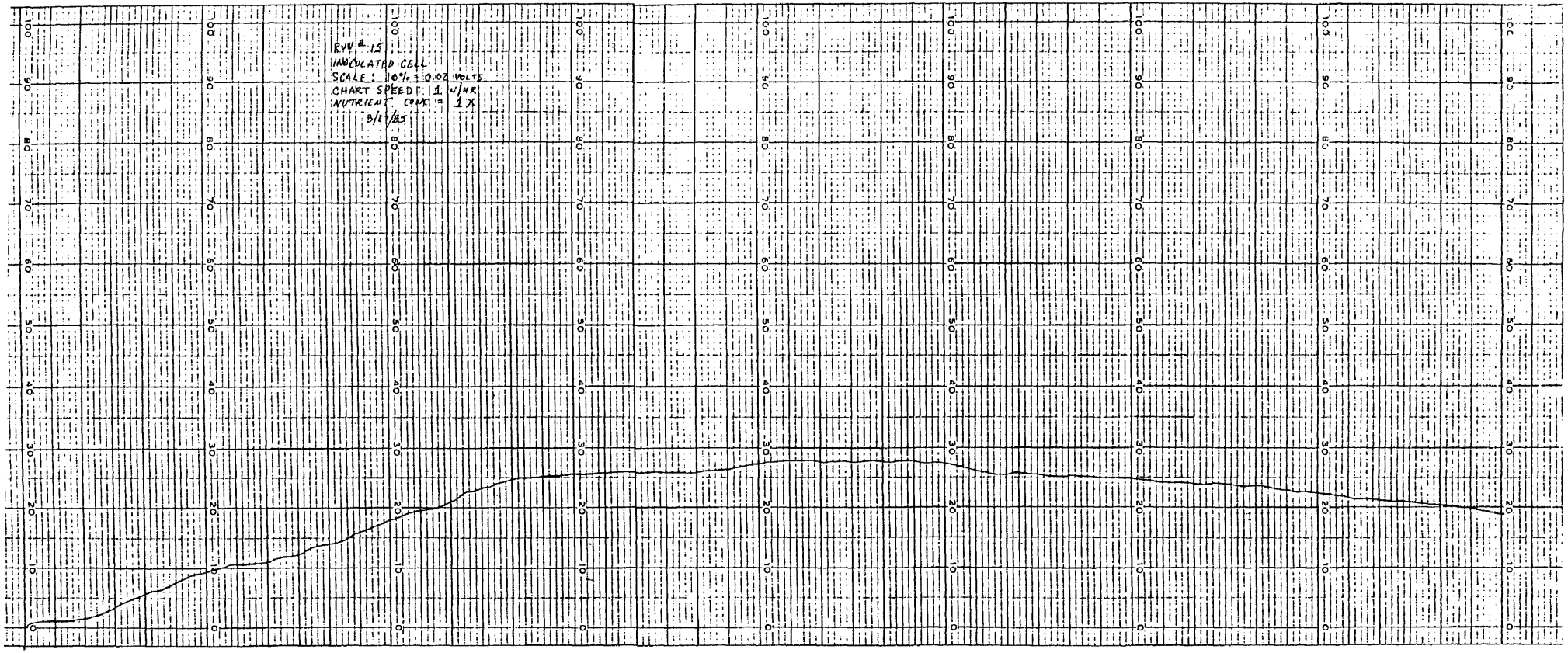


CHART 11
INOCULATED FUEL CELL OUTPUT
INITIAL CONC.

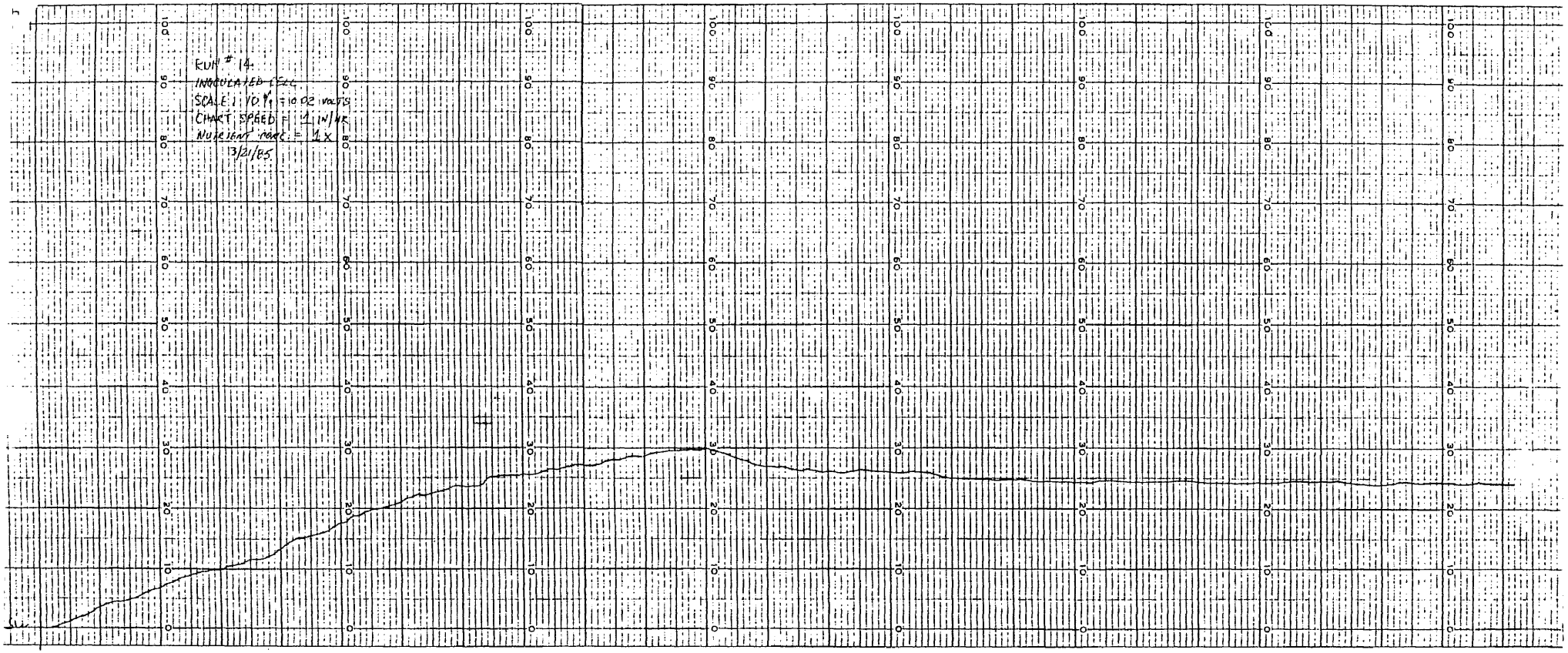


CHART 12
INOCULATED FUEL CELL OUTPUT
INITIAL CONC.

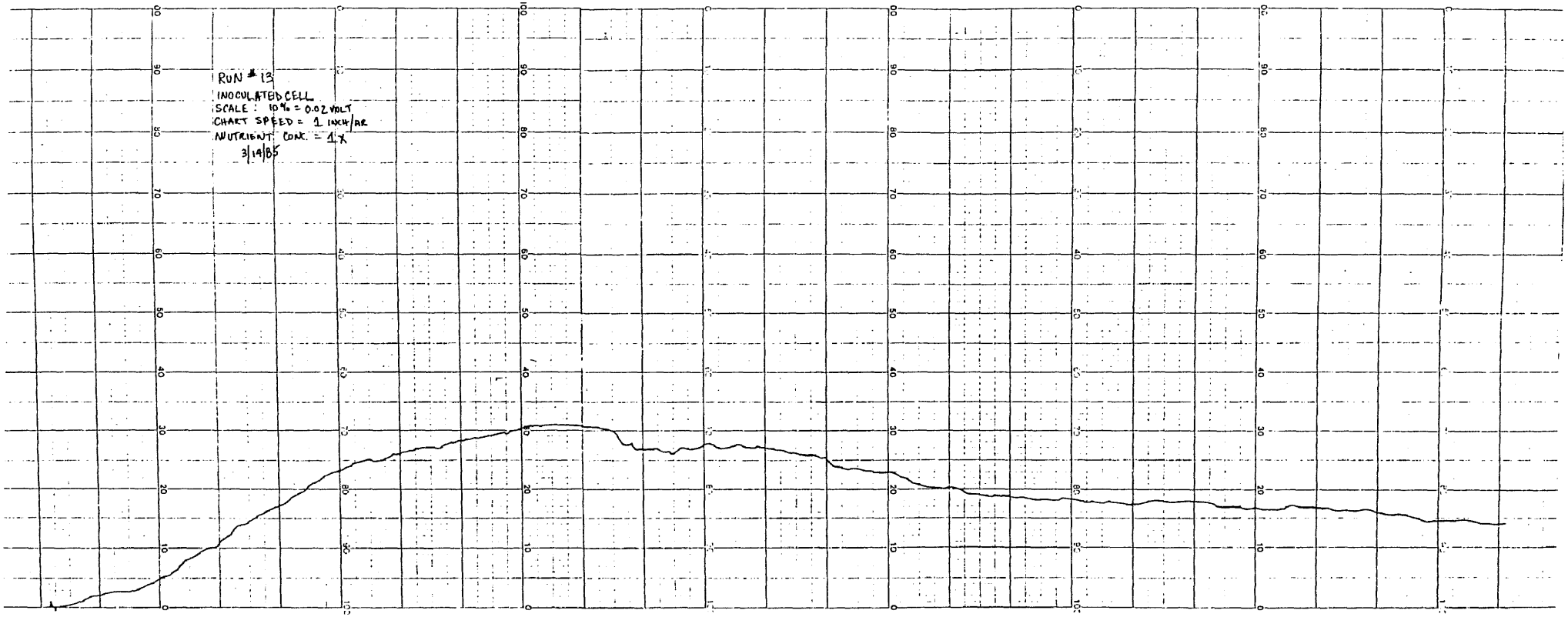


CHART 13
UNINOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

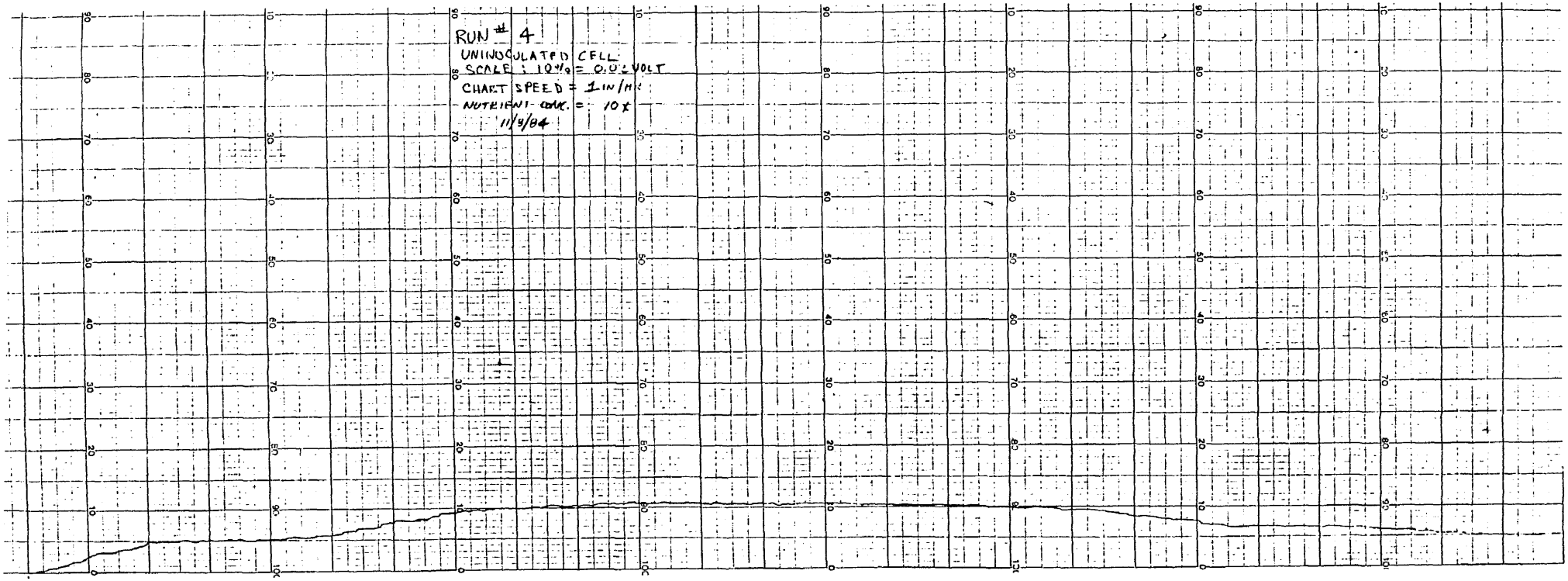


CHART 14
ININOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

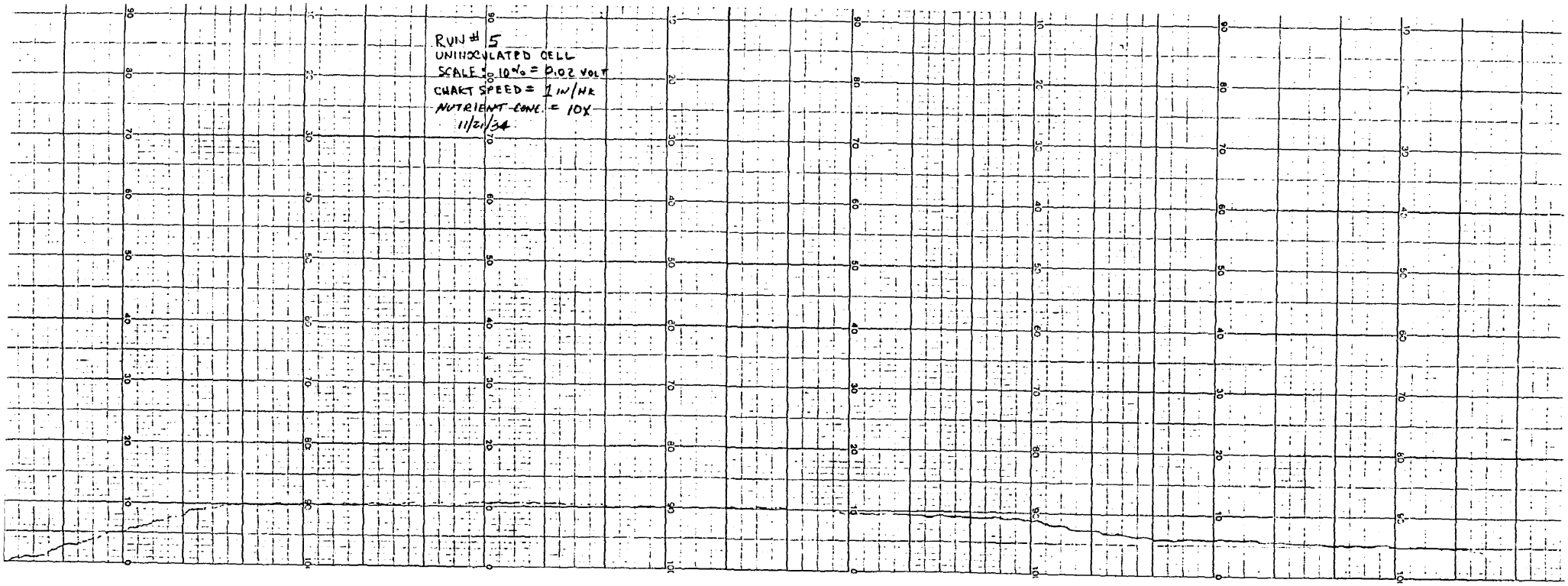


CHART 15
UNINOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

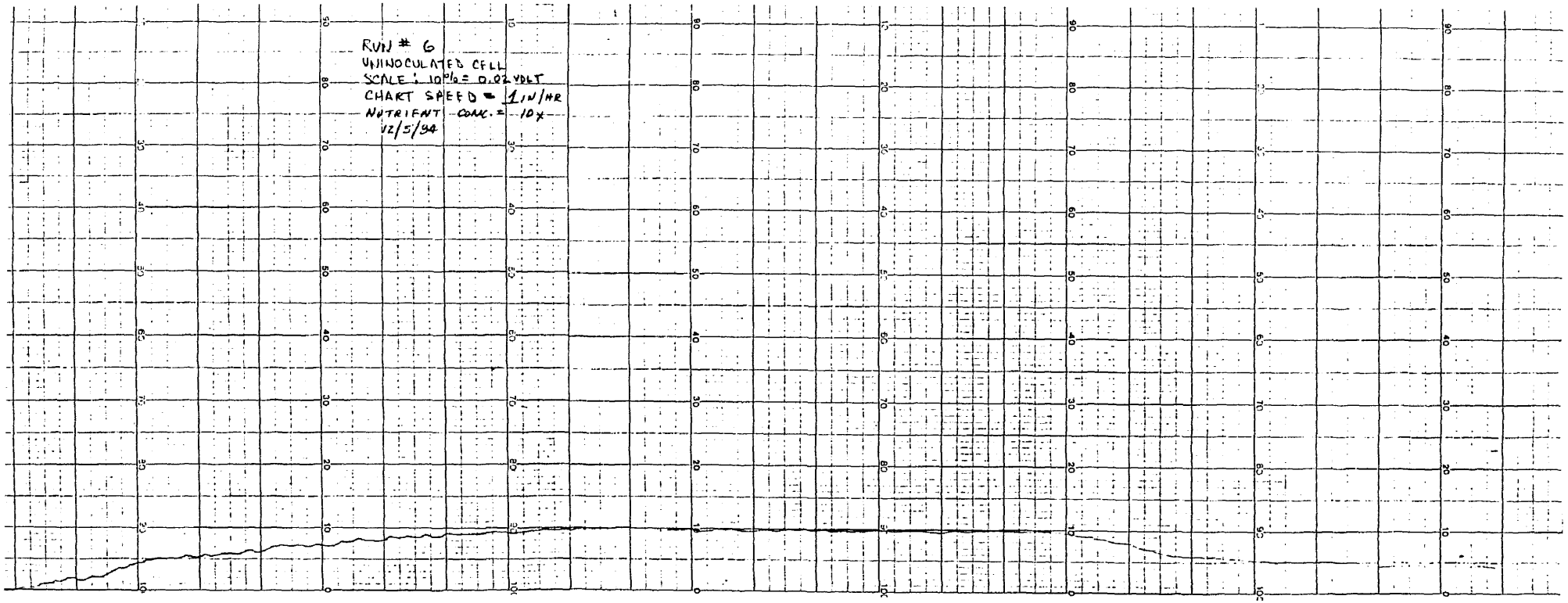


CHART 16
UNINOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

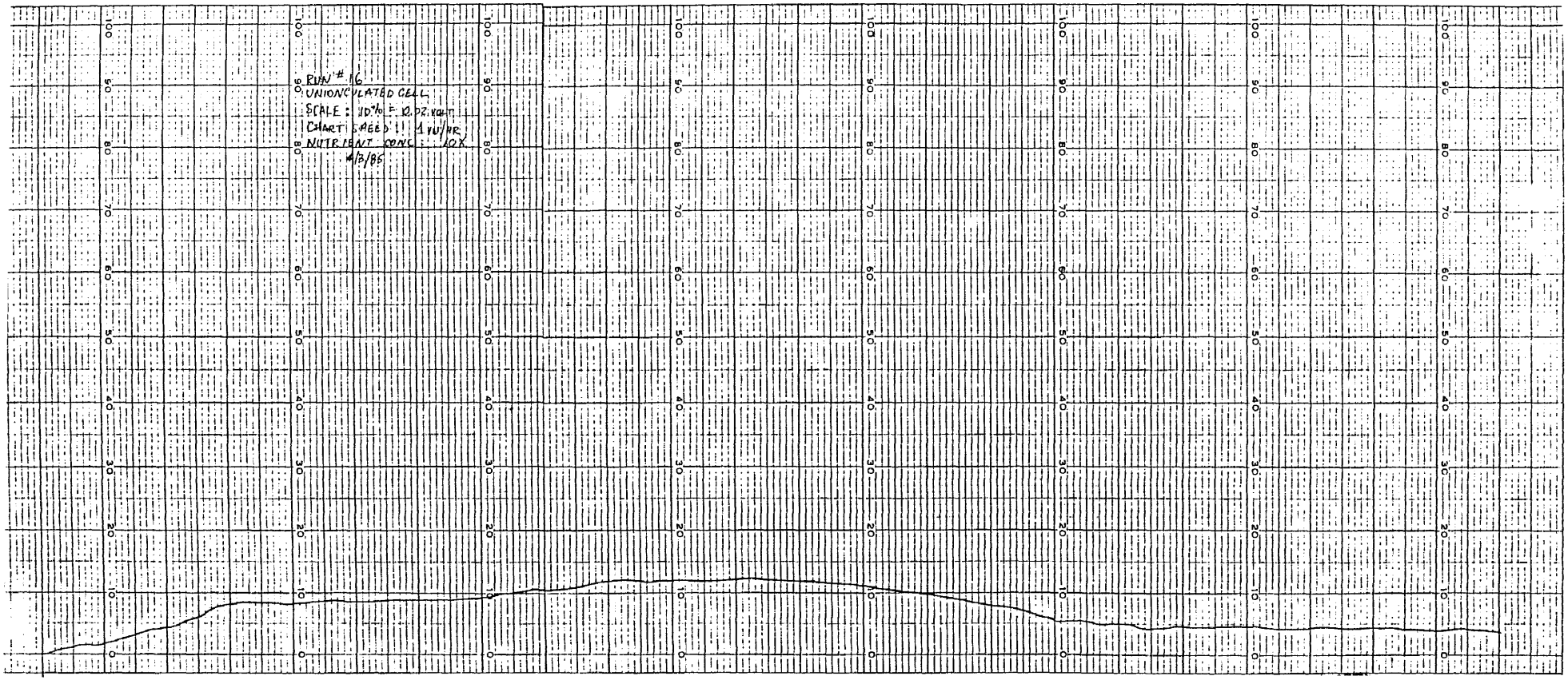


CHART 17
UNINOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

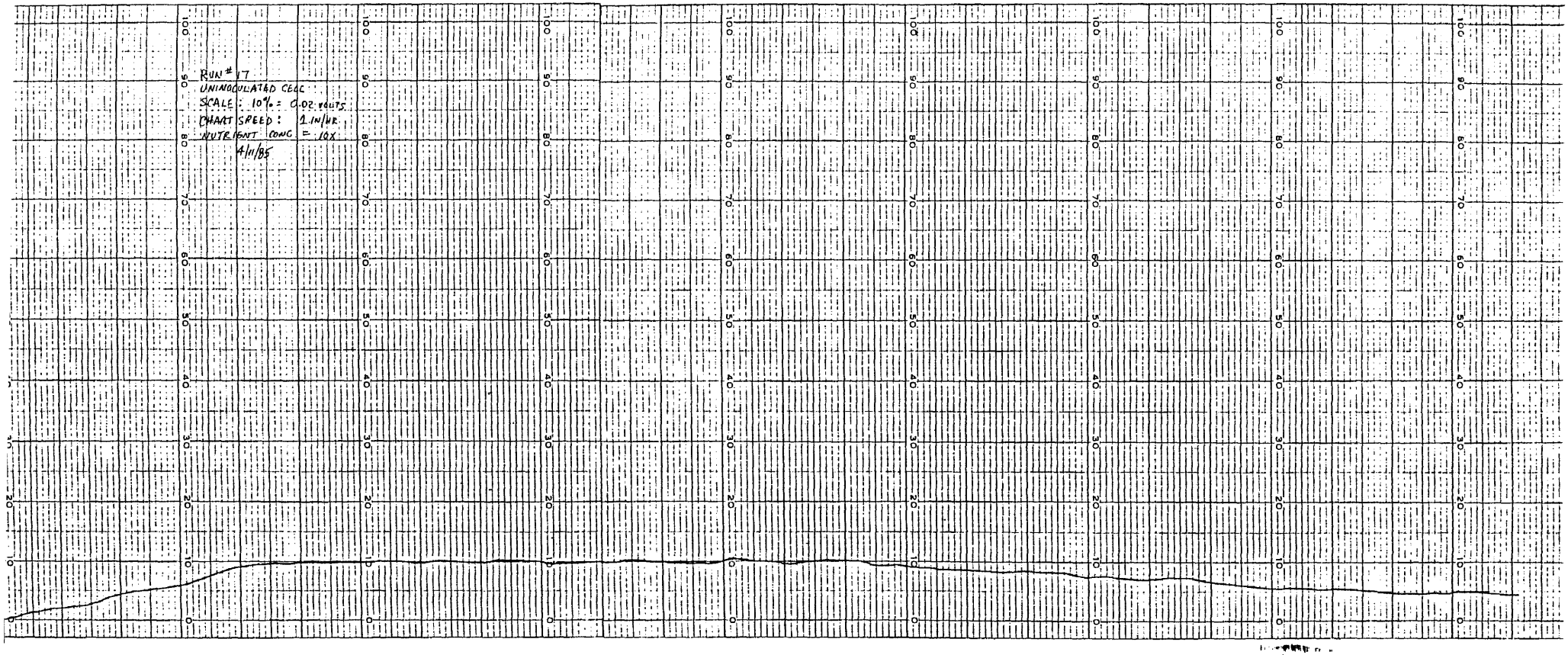


CHART 18
UNINOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

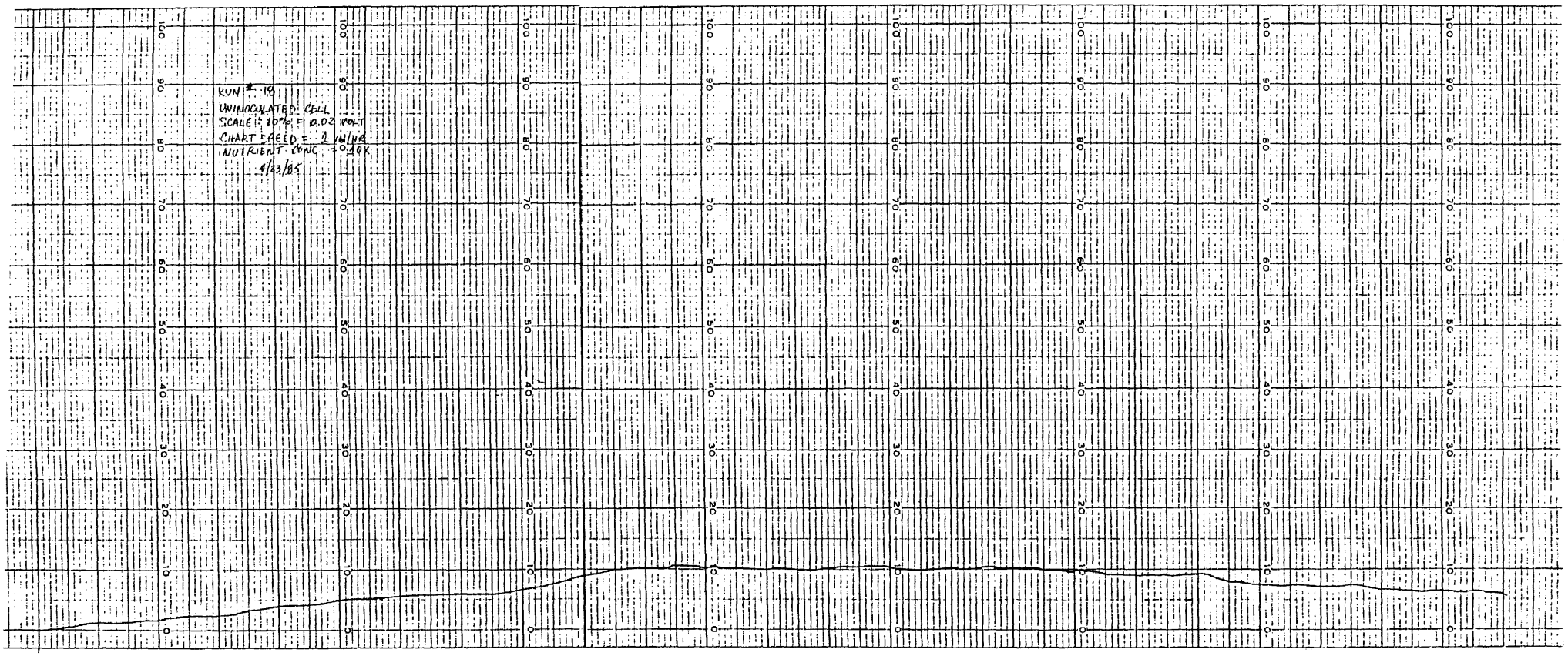


CHART 19
INOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

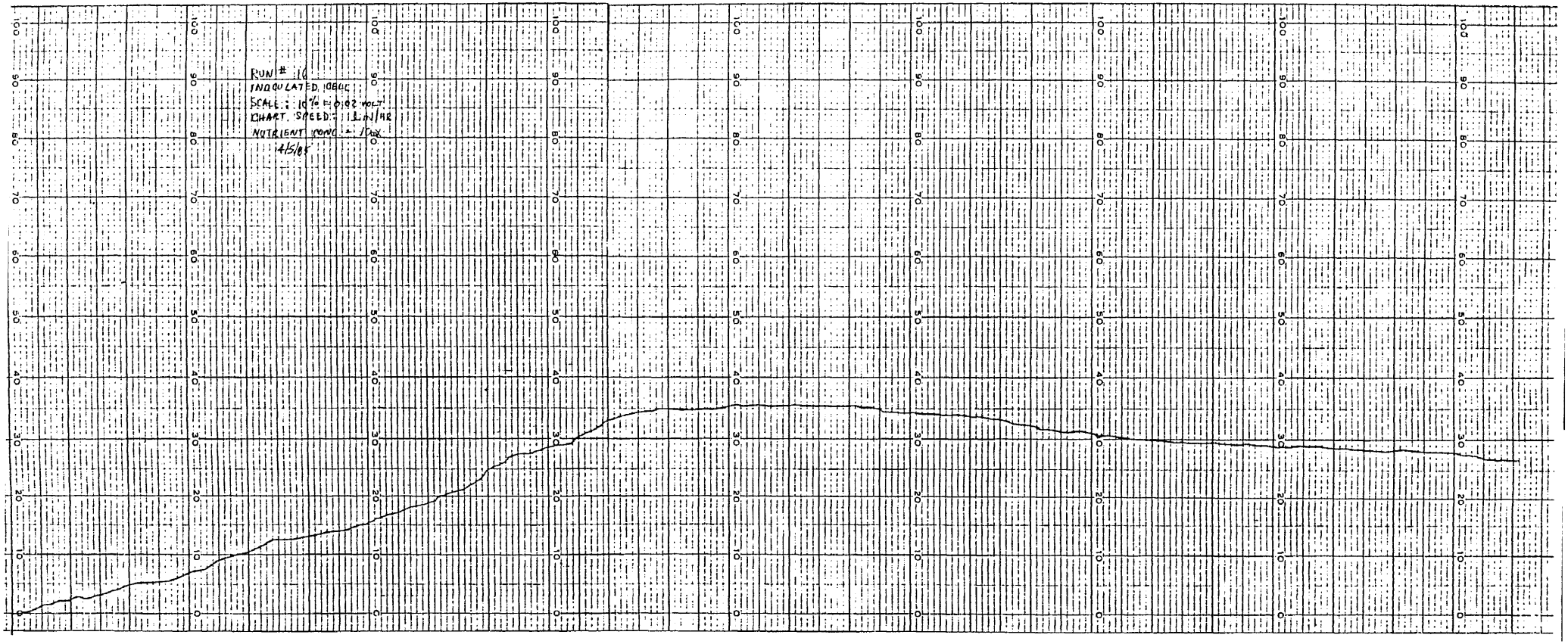


CHART 20
INOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

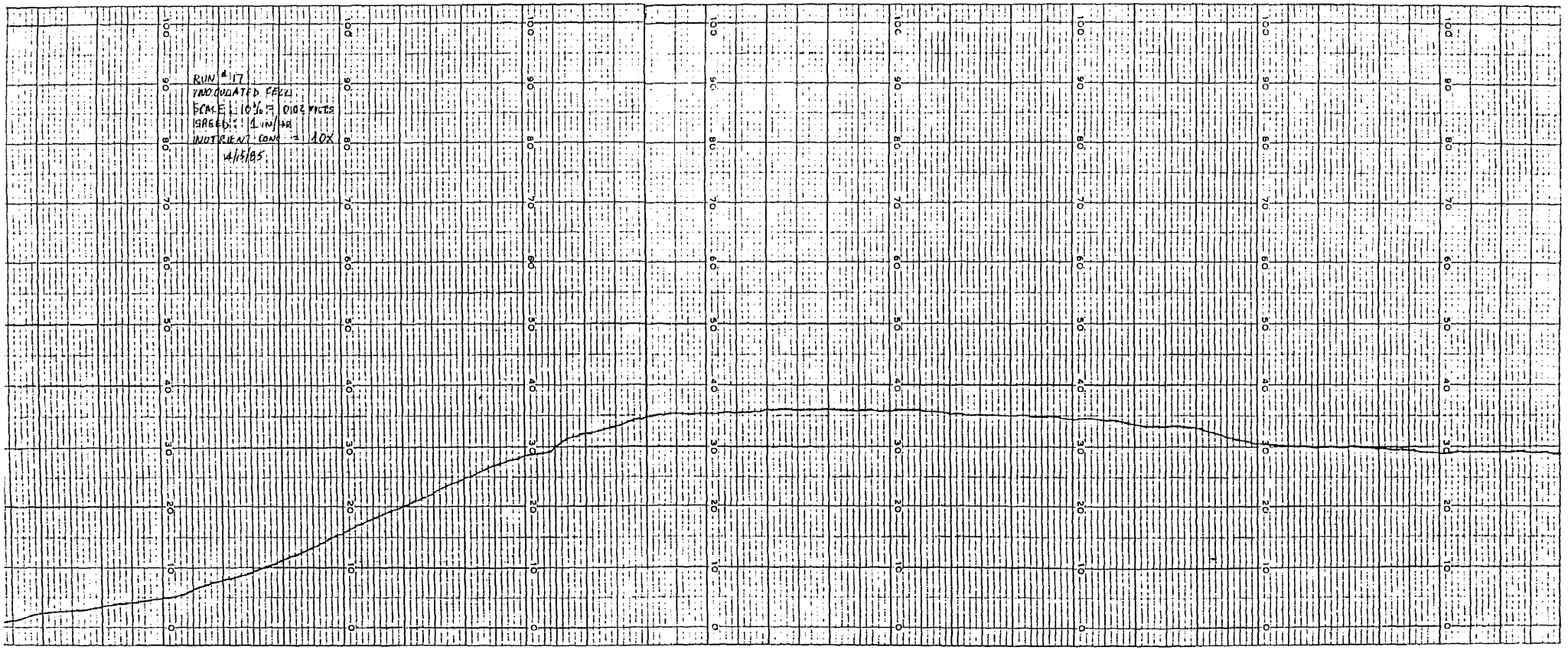


CHART 21
INOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

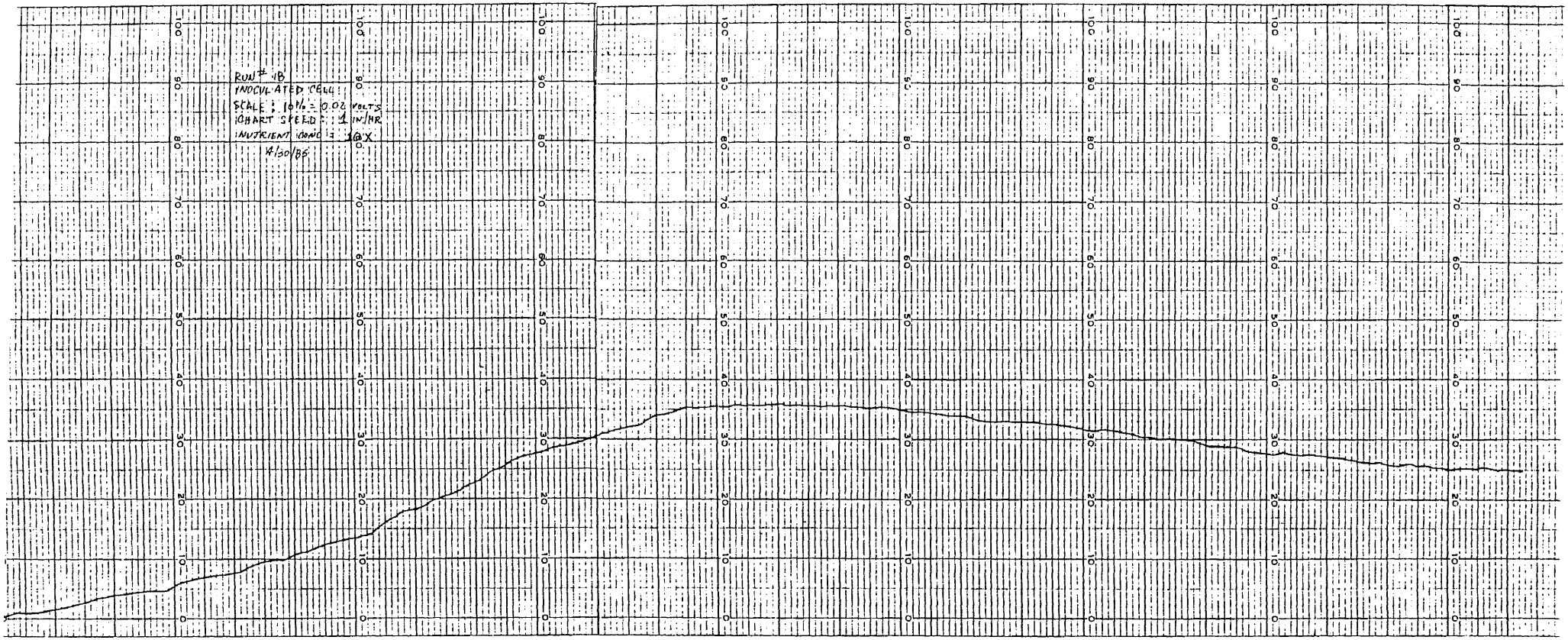


CHART 22
INOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

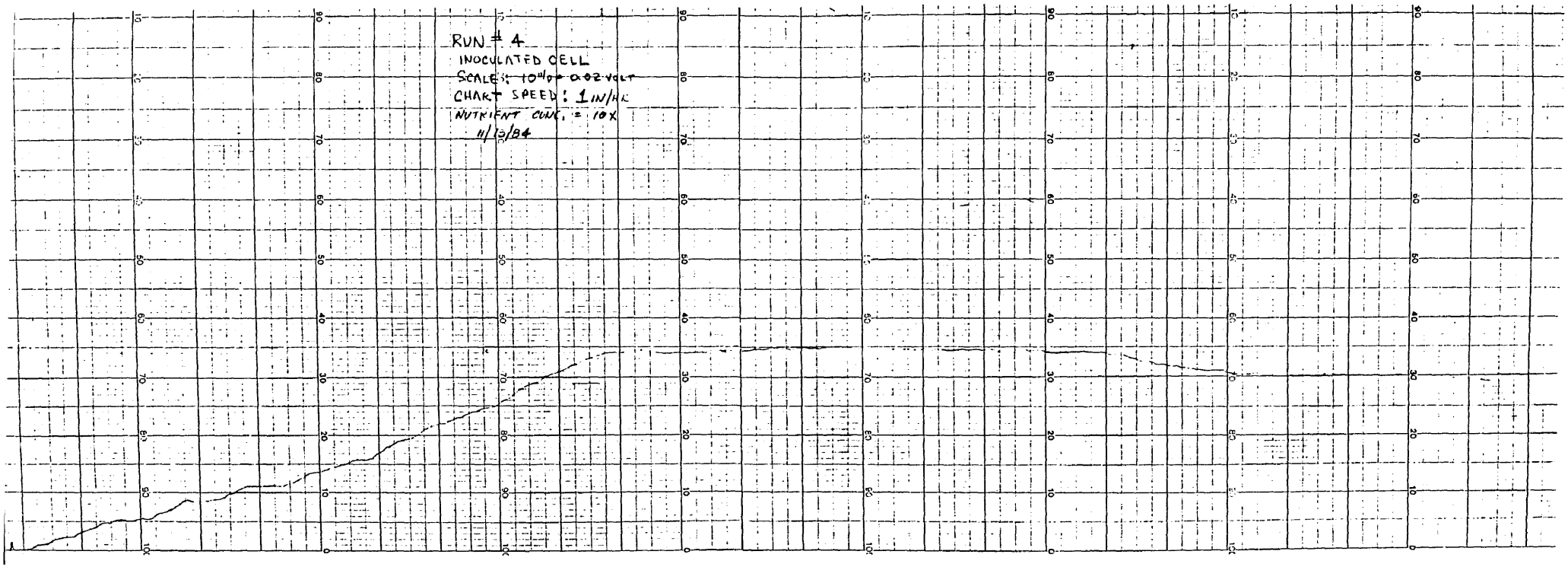


CHART 23
INOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

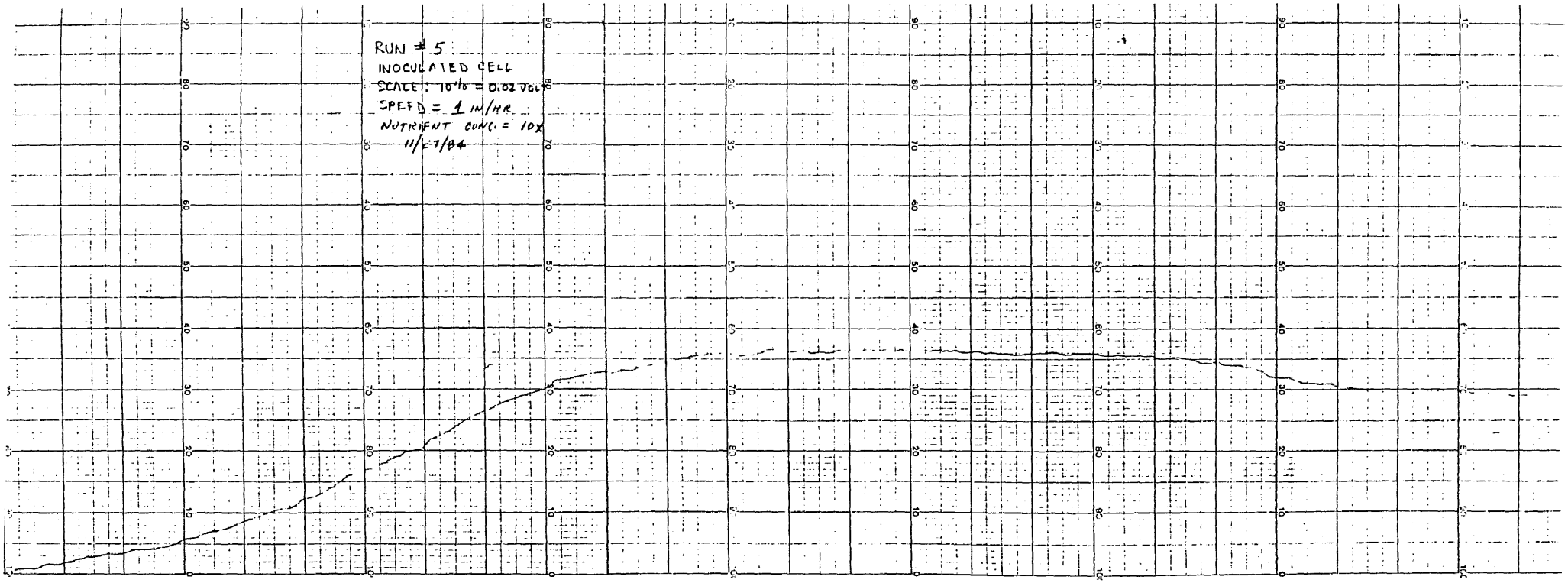


CHART 24
INOCULATED FUEL CELL OUTPUT
10 FOLD CONC.

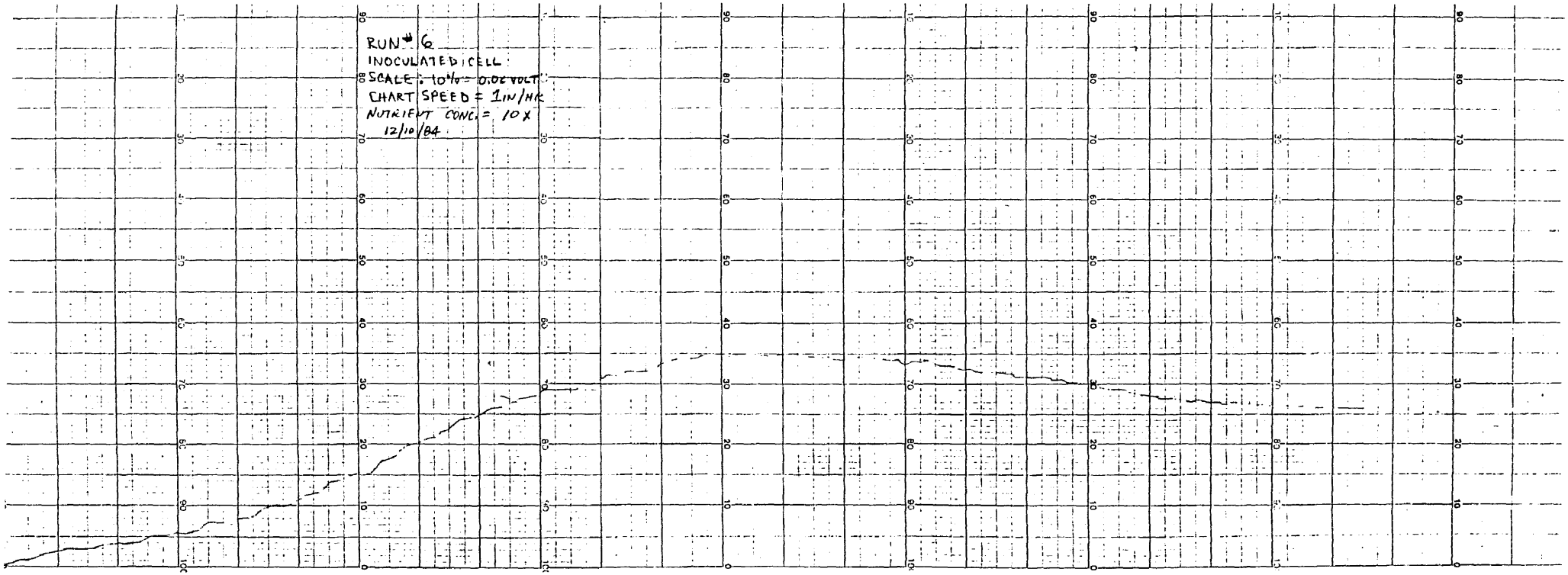


CHART 25
UNINOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

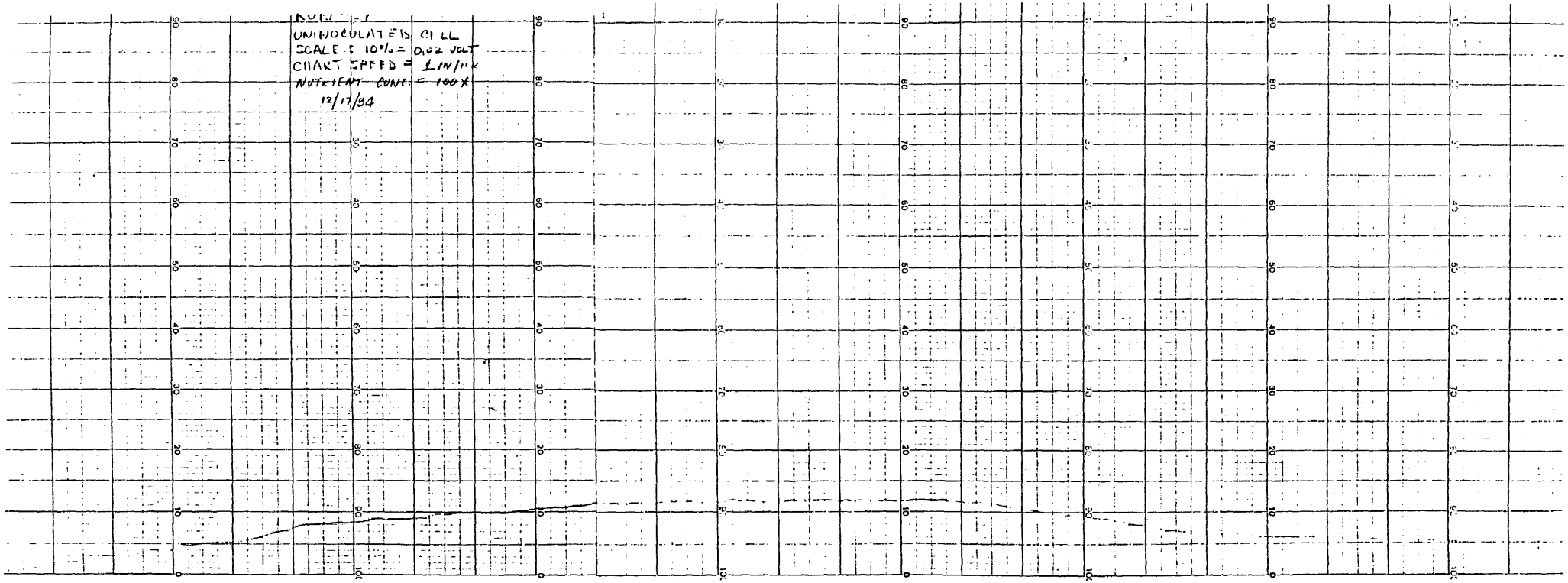


CHART 26
UNINOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

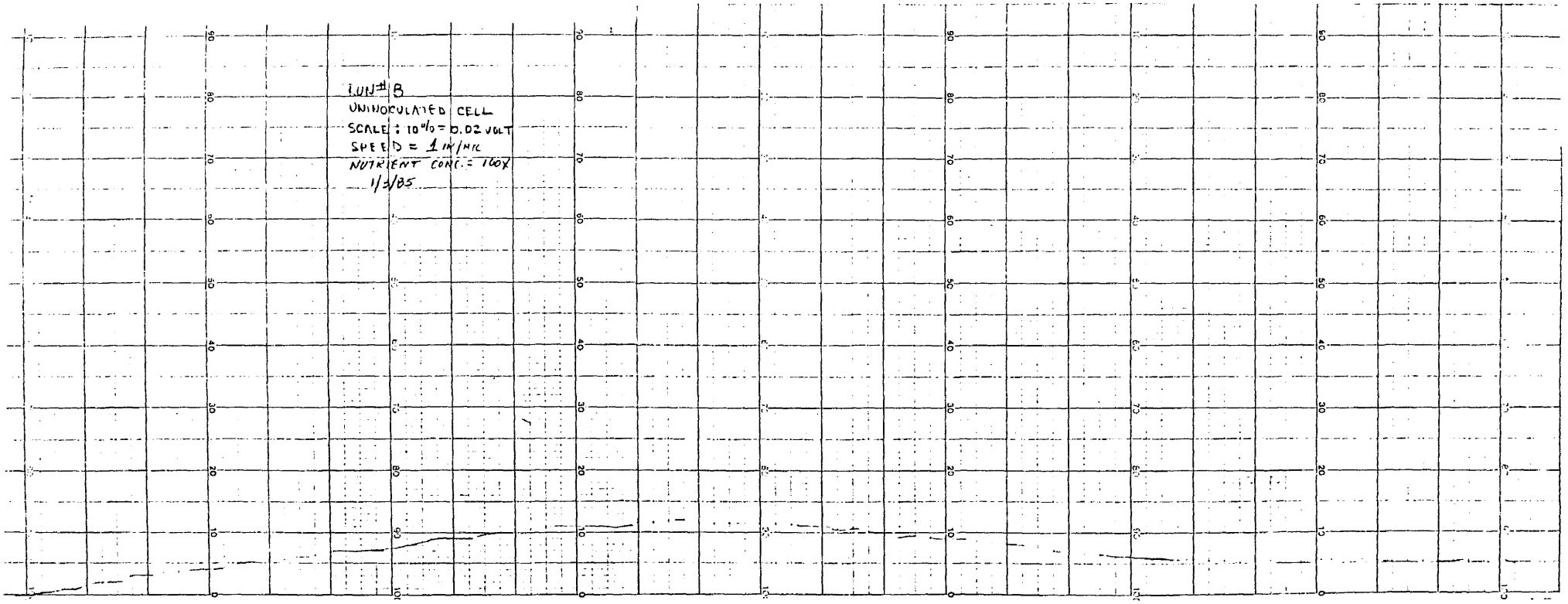


CHART 27
UNINOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

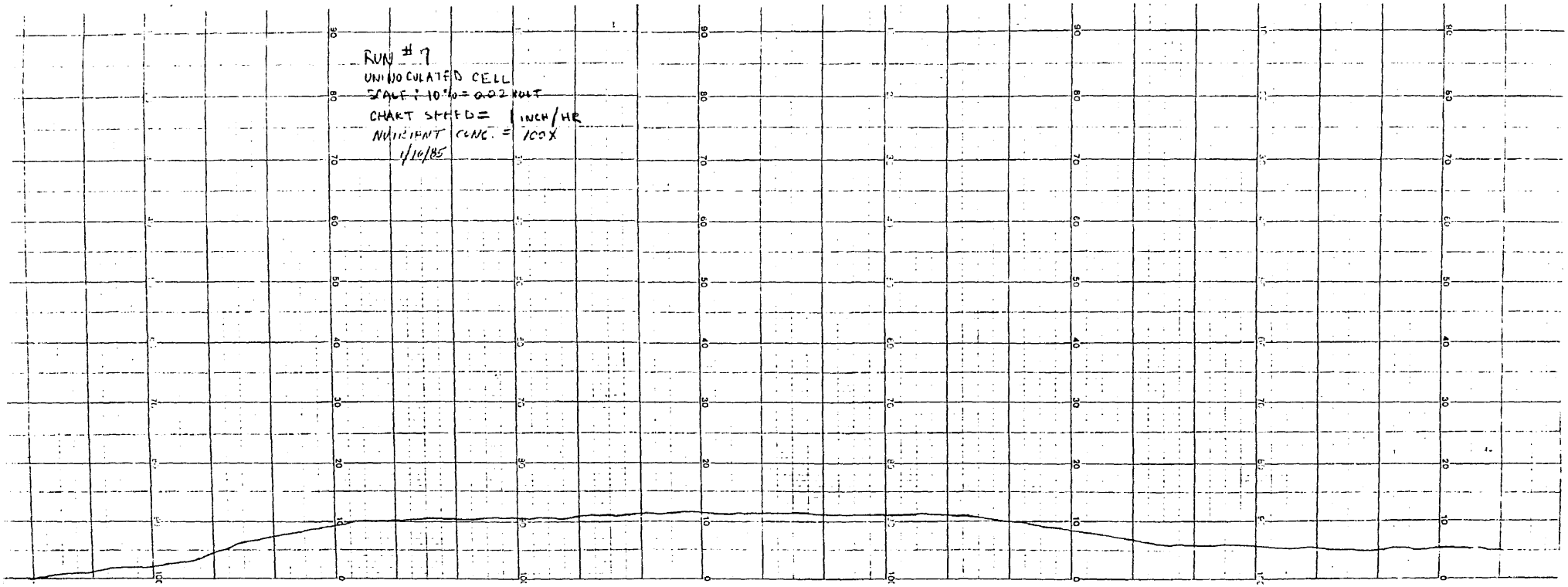


CHART 28
UNINOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

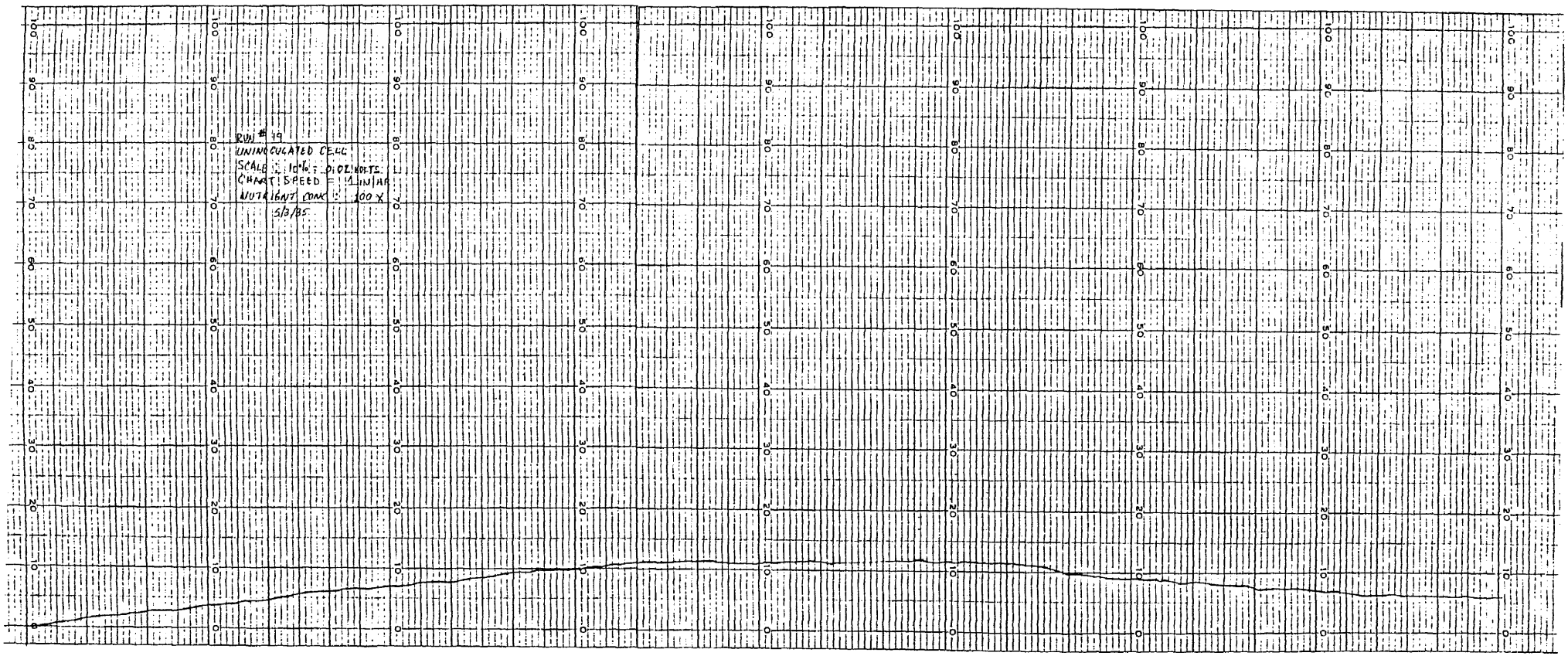


CHART 29
UNINOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

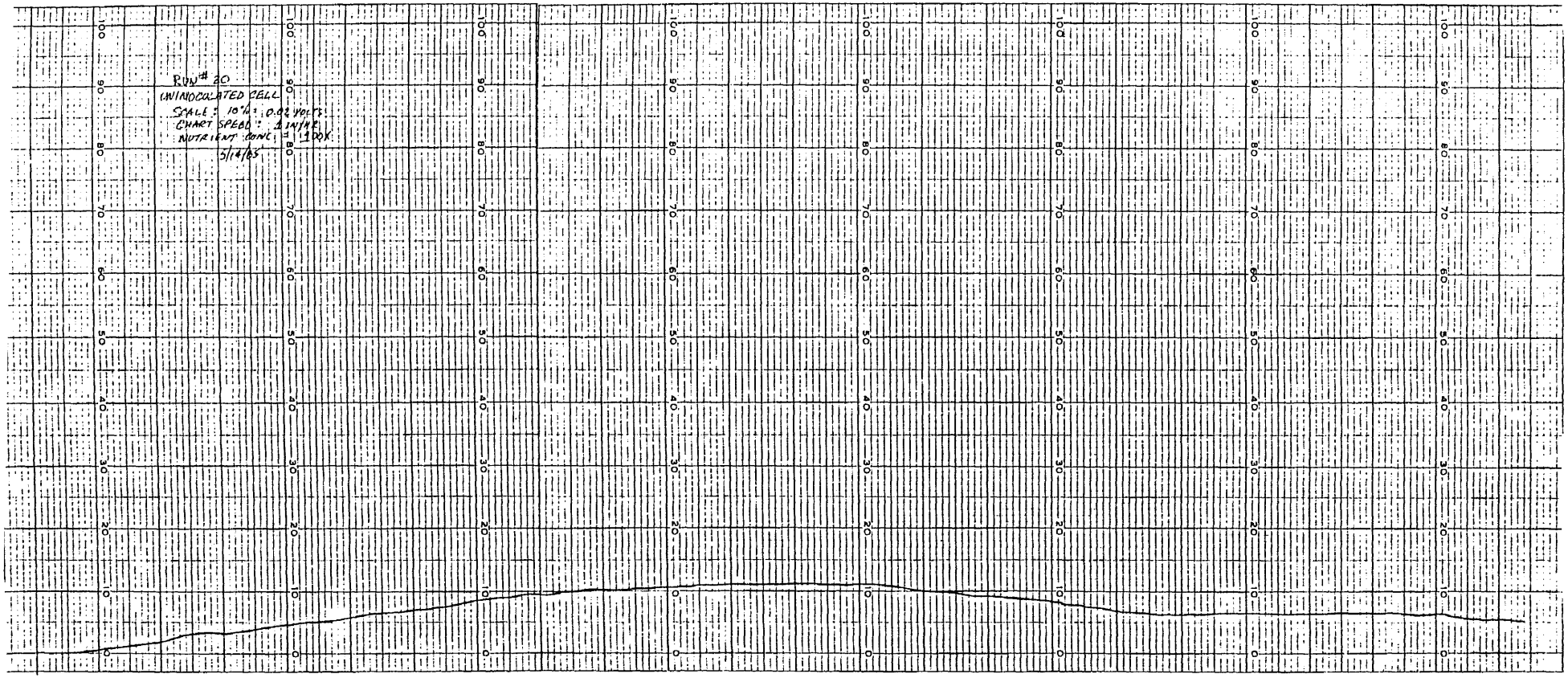


CHART 30
UNINOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

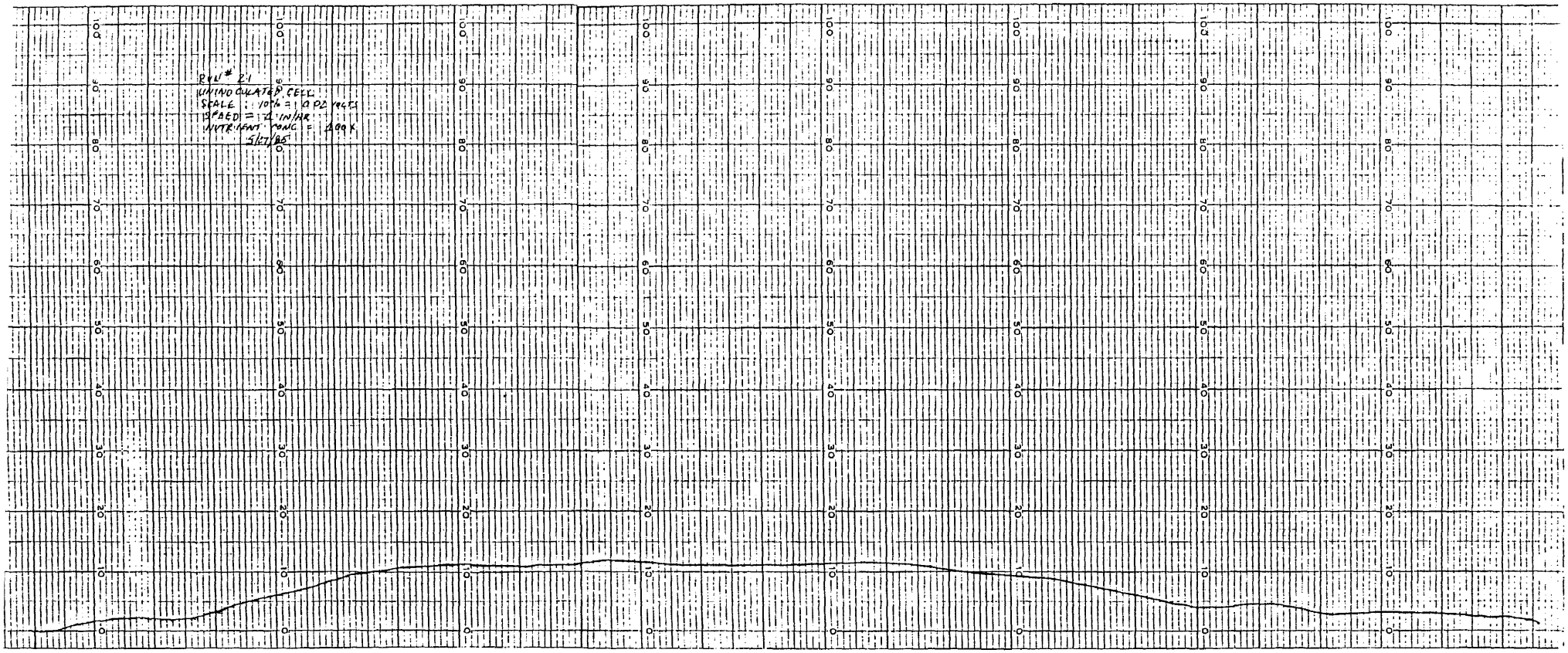


CHART 31
INOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

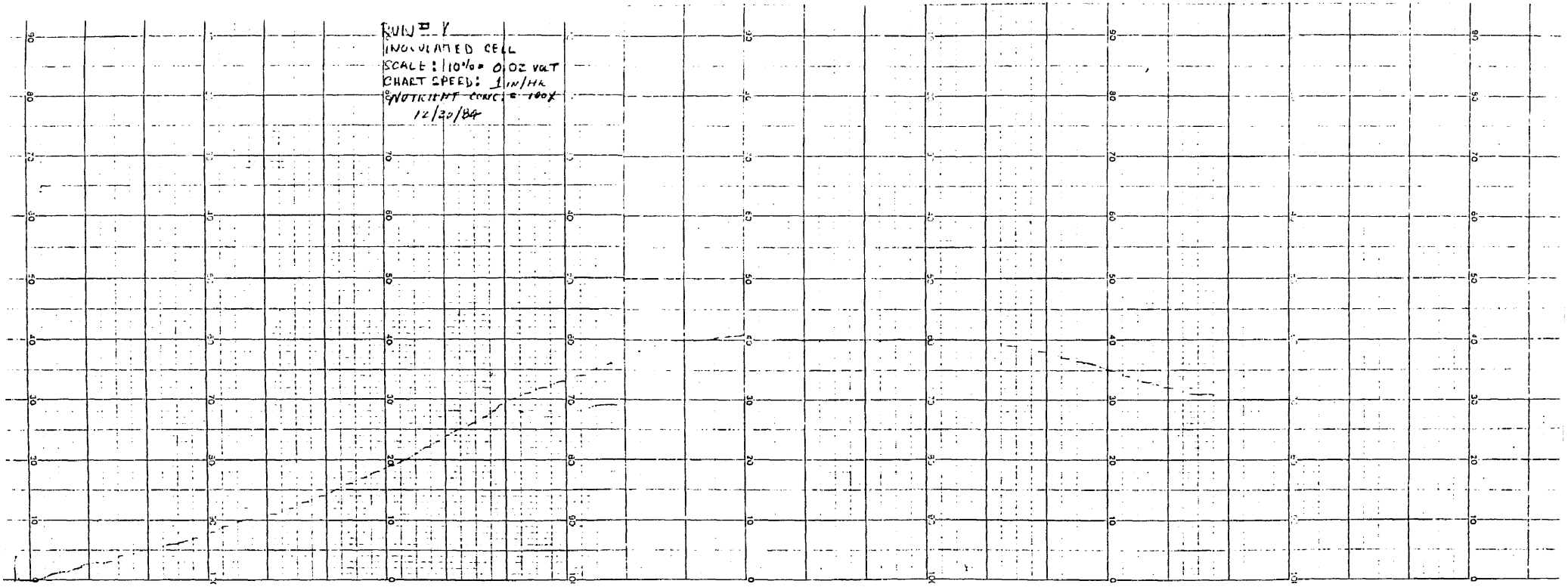


CHART 32
INOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

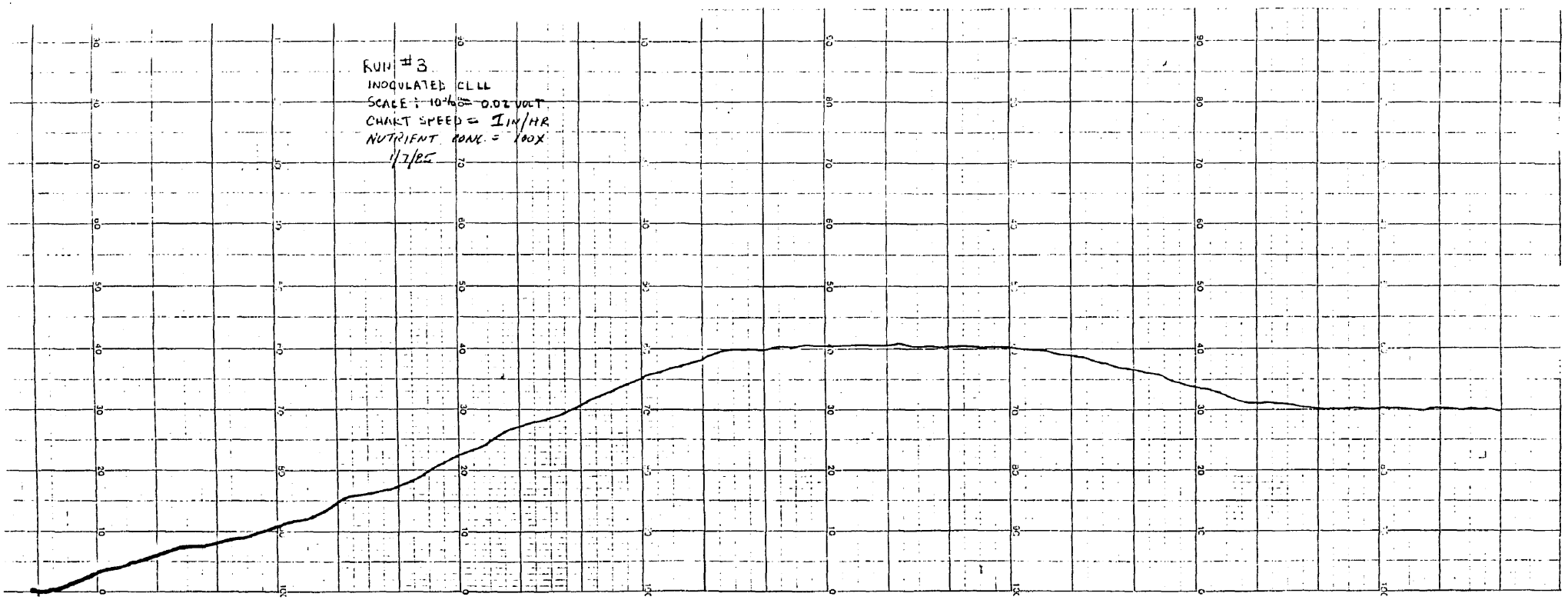


CHART 33
INOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

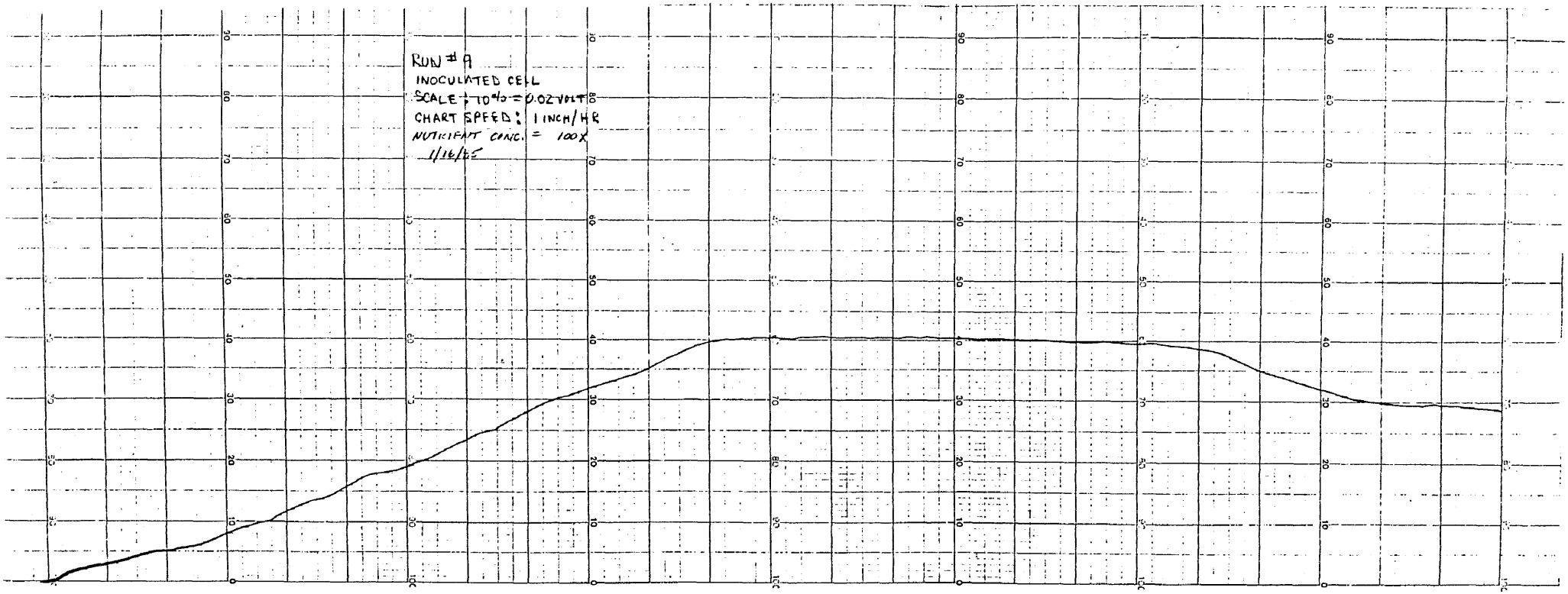


CHART 34
INOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

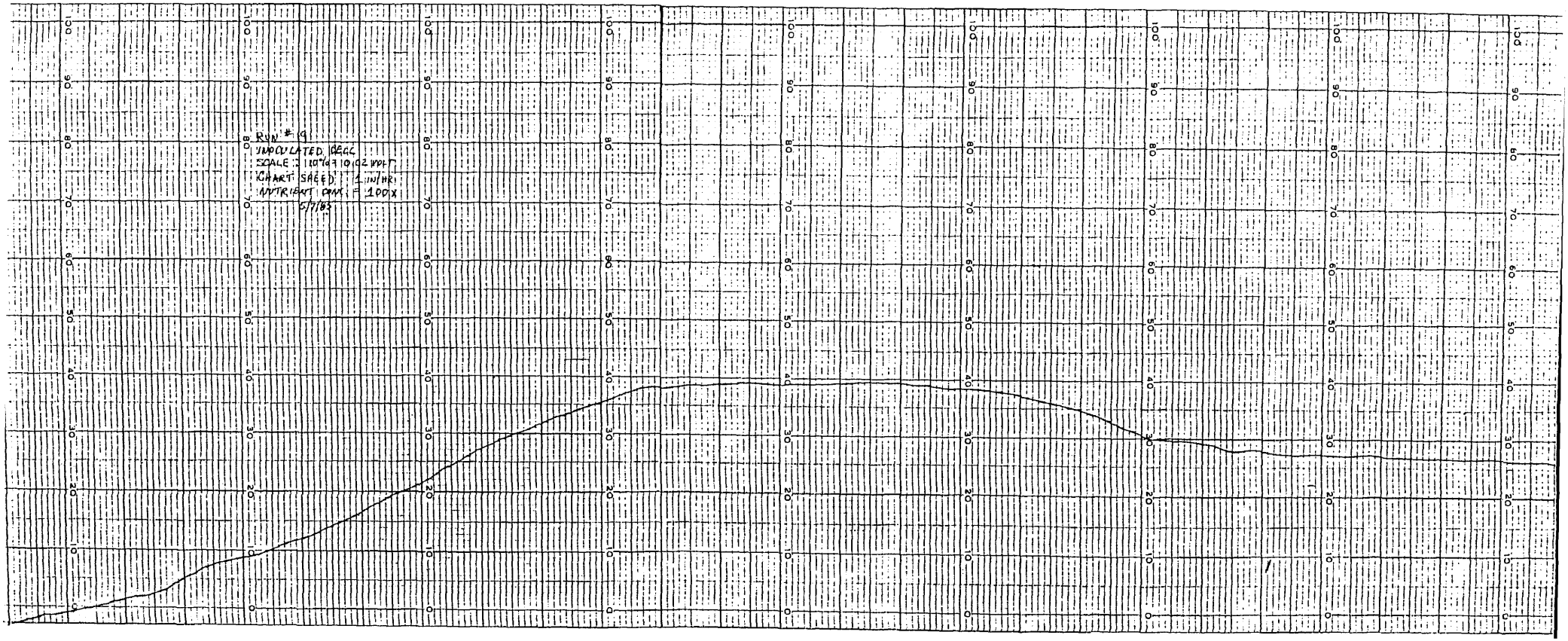


CHART 35
INOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

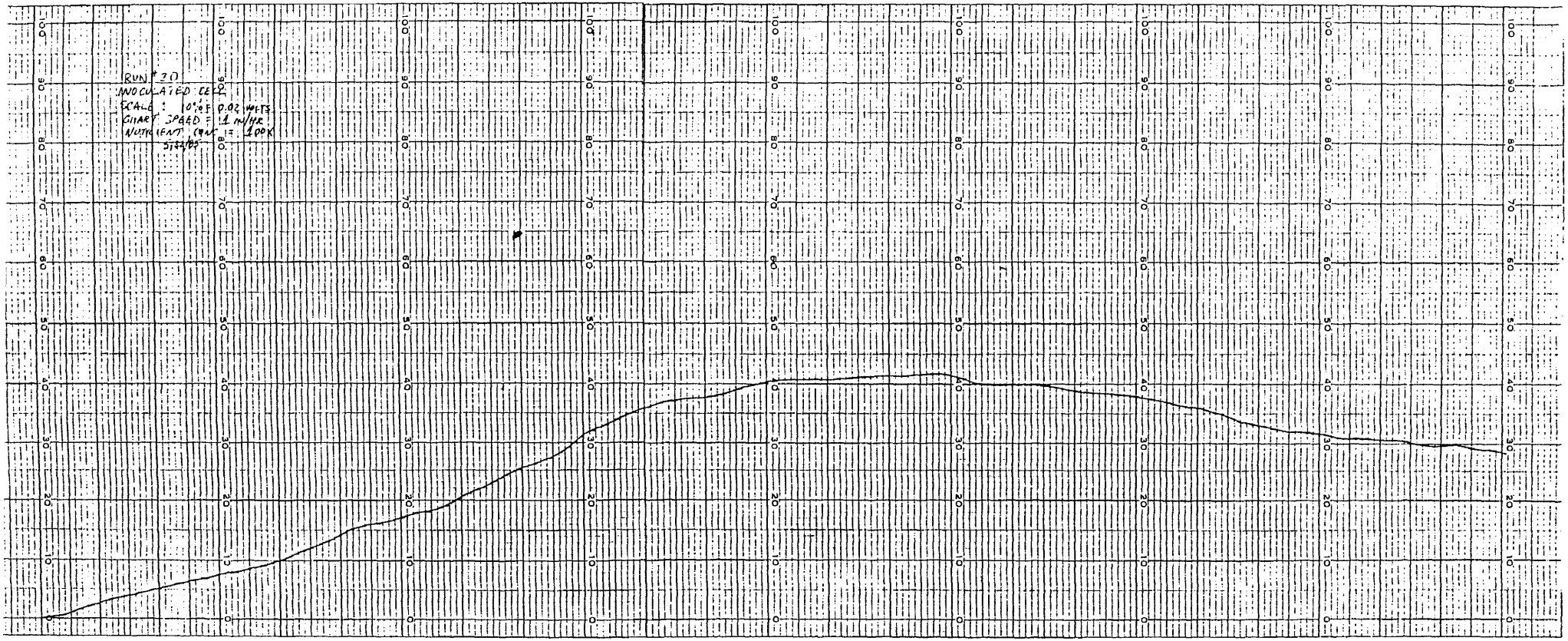


CHART 36
INOCULATED FUEL CELL OUTPUT
100 FOLD CONC.

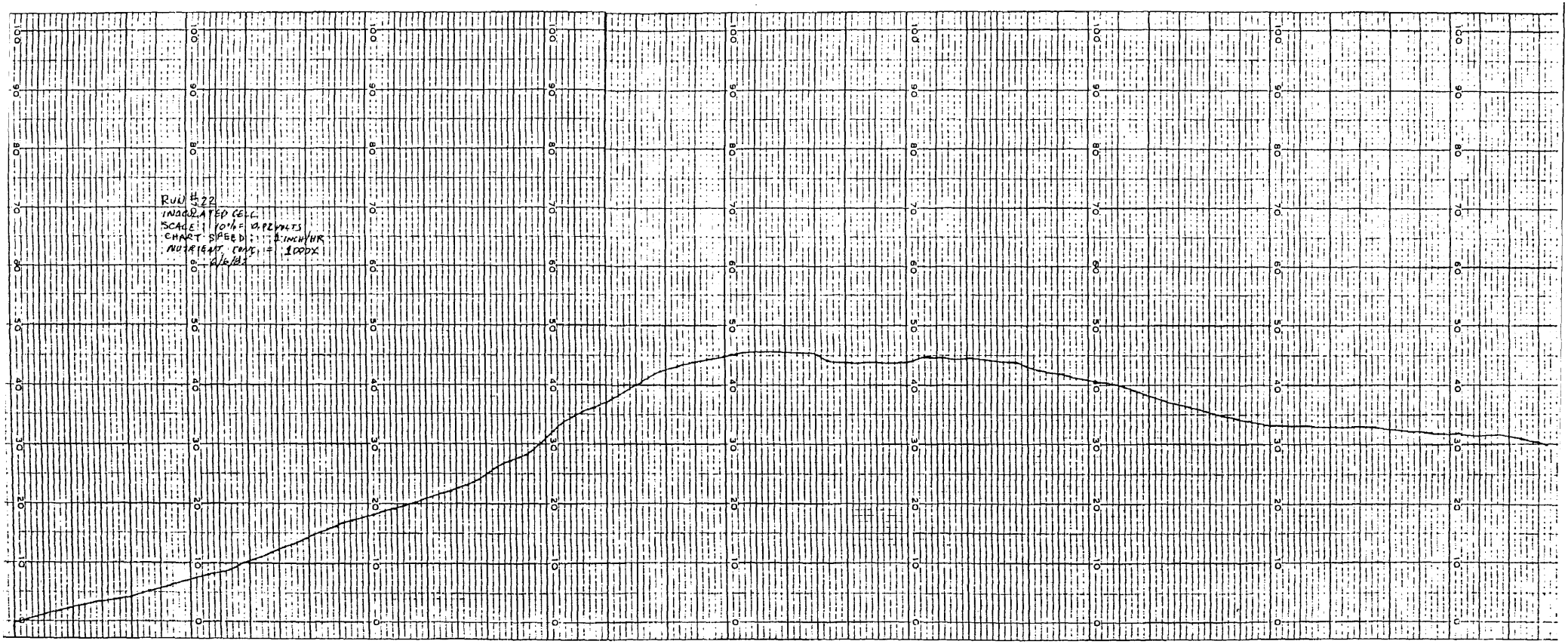


CHART 37
UNINOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

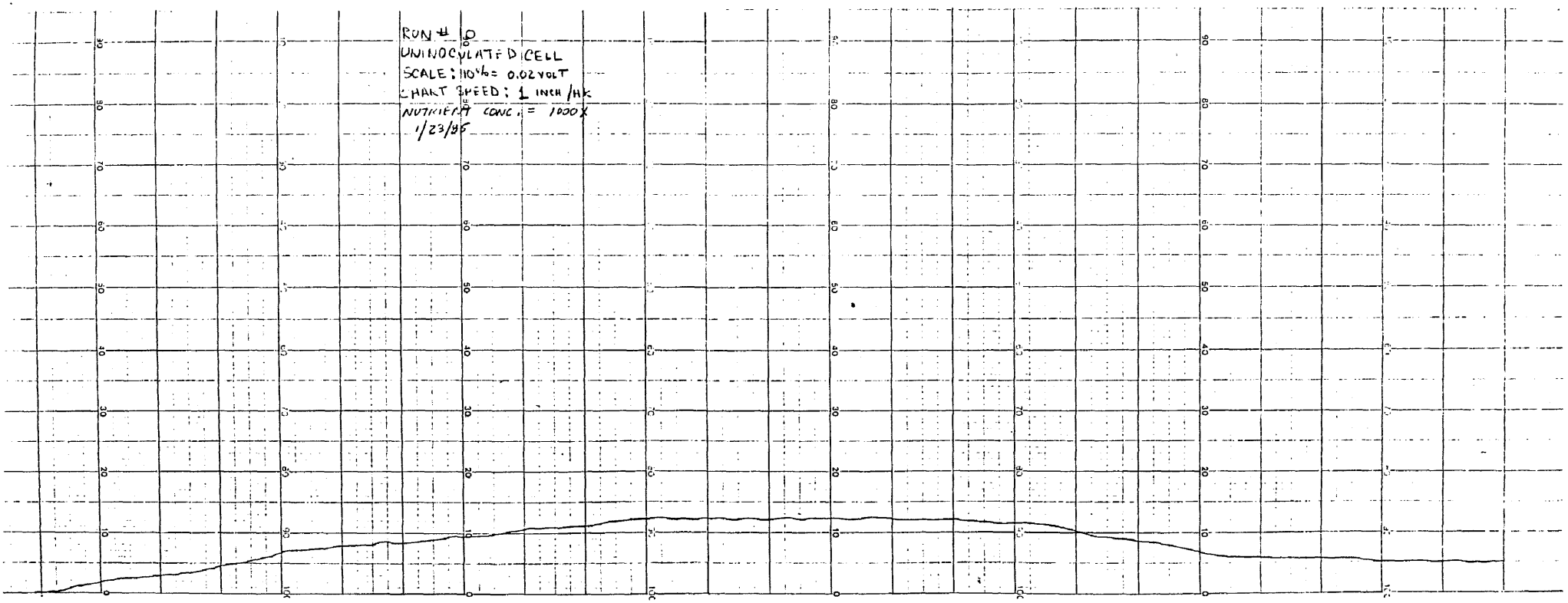


CHART 38
UNINOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

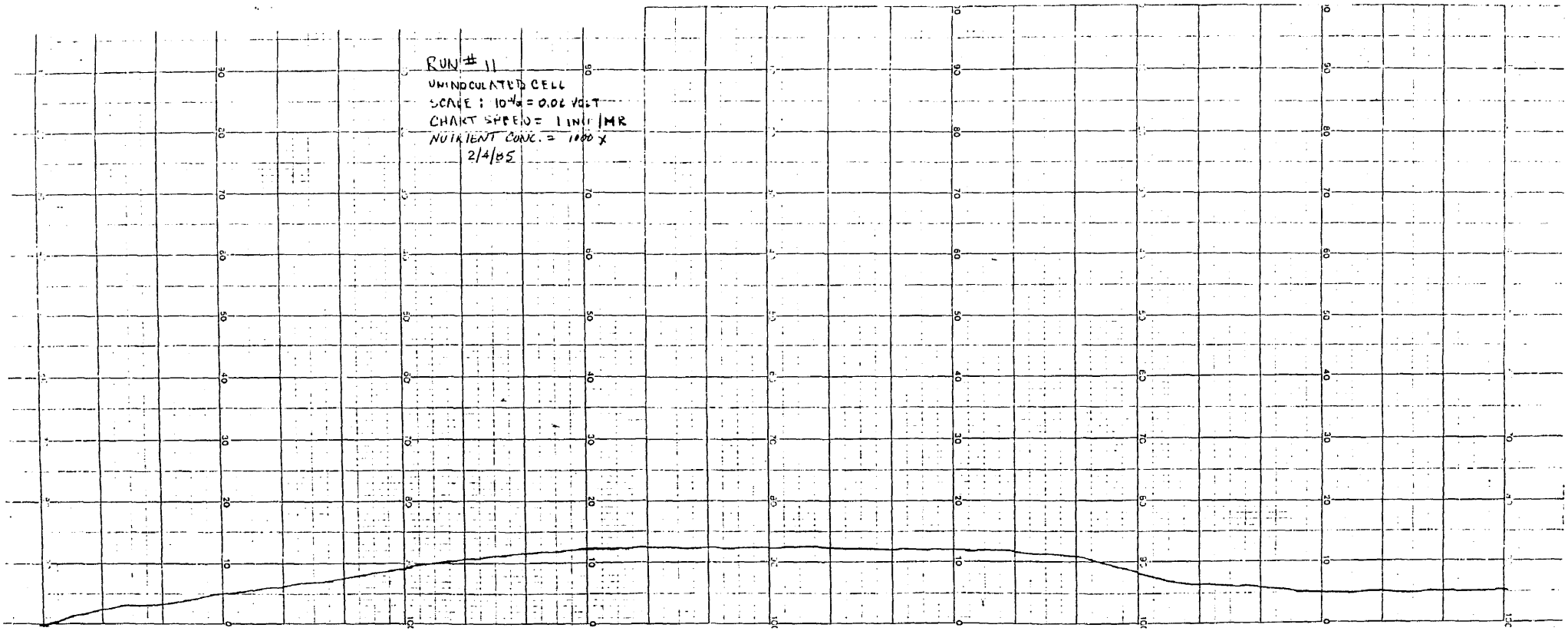


CHART 39
UNINOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

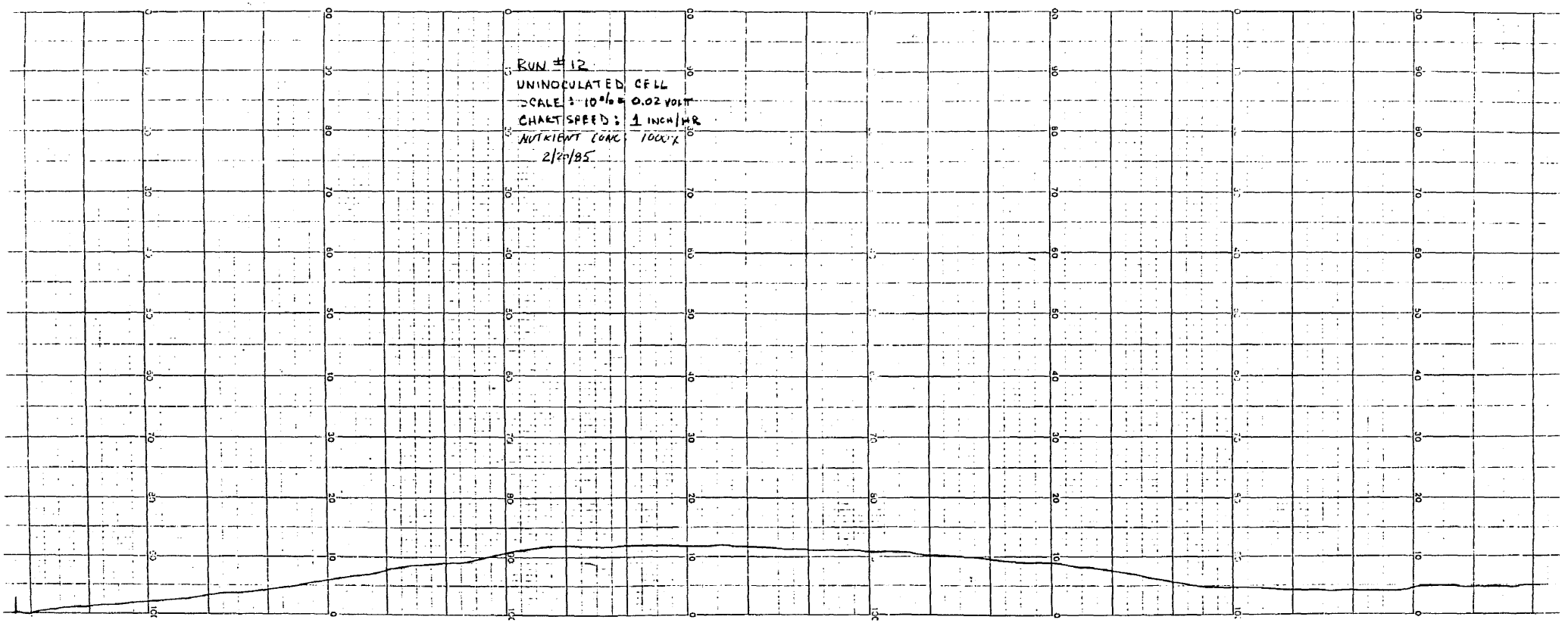


CHART 40
UNINOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

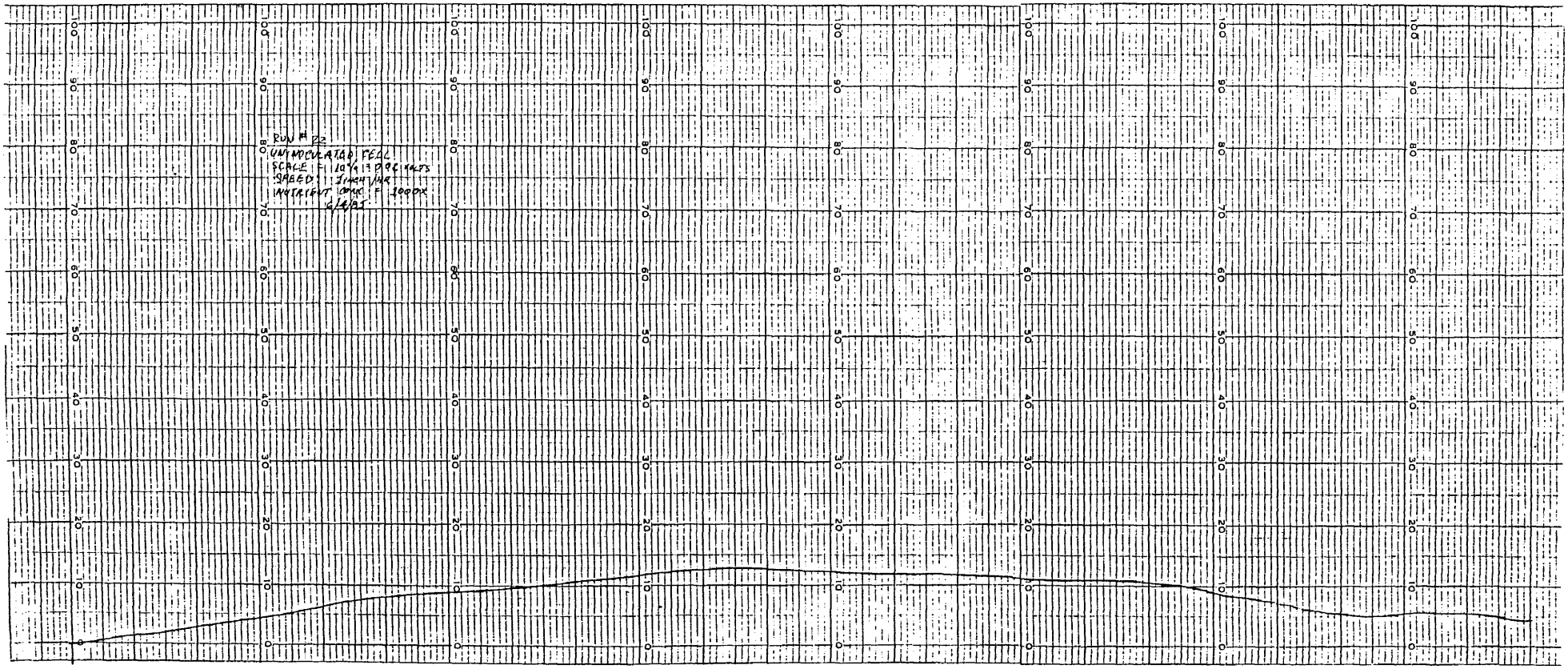


CHART 41
UNINOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

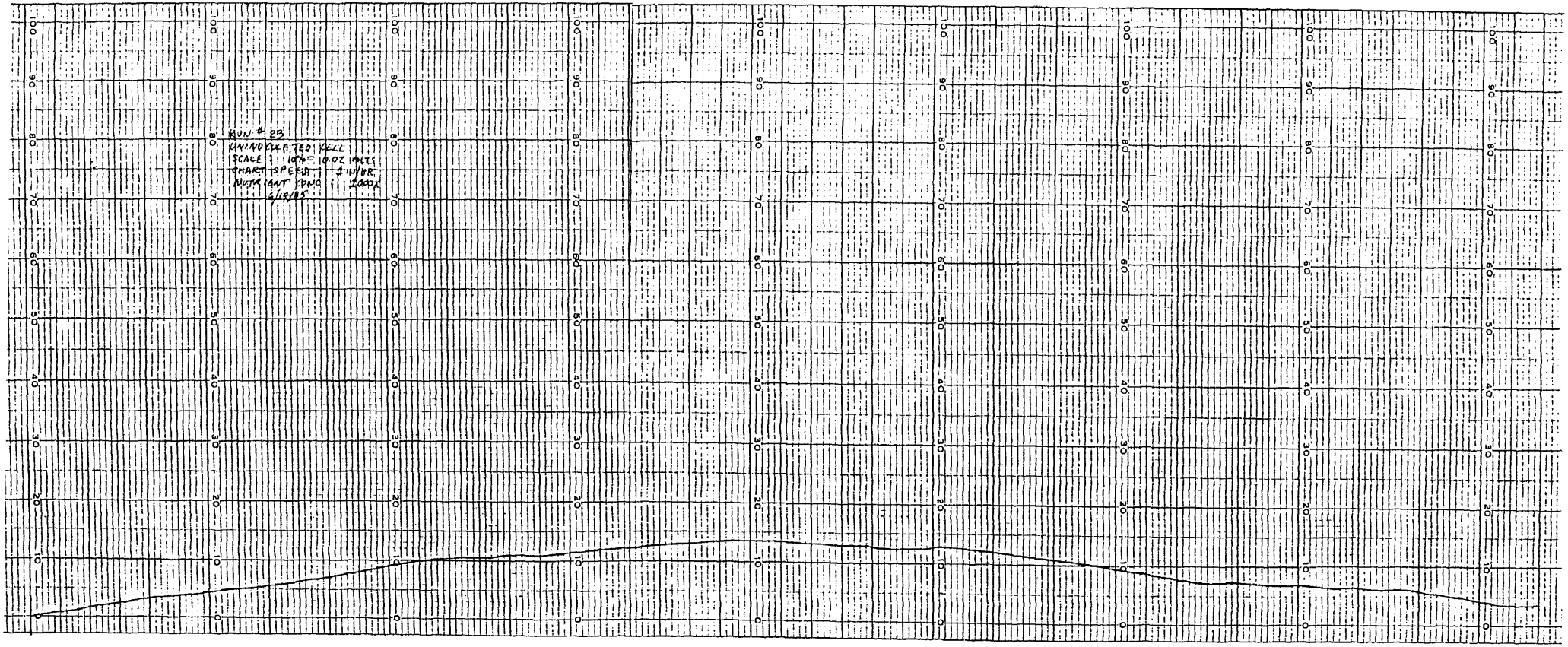


CHART 42
UNINOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

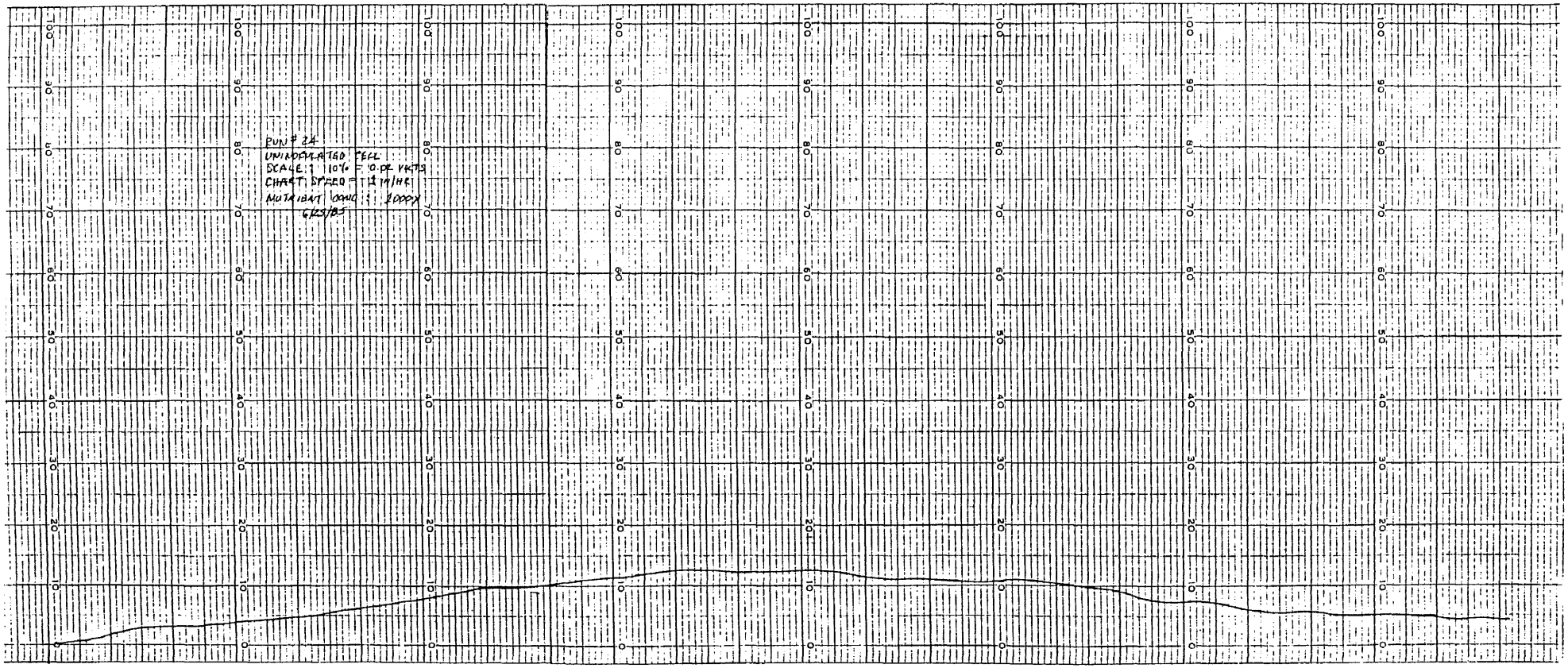


CHART 43
INOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

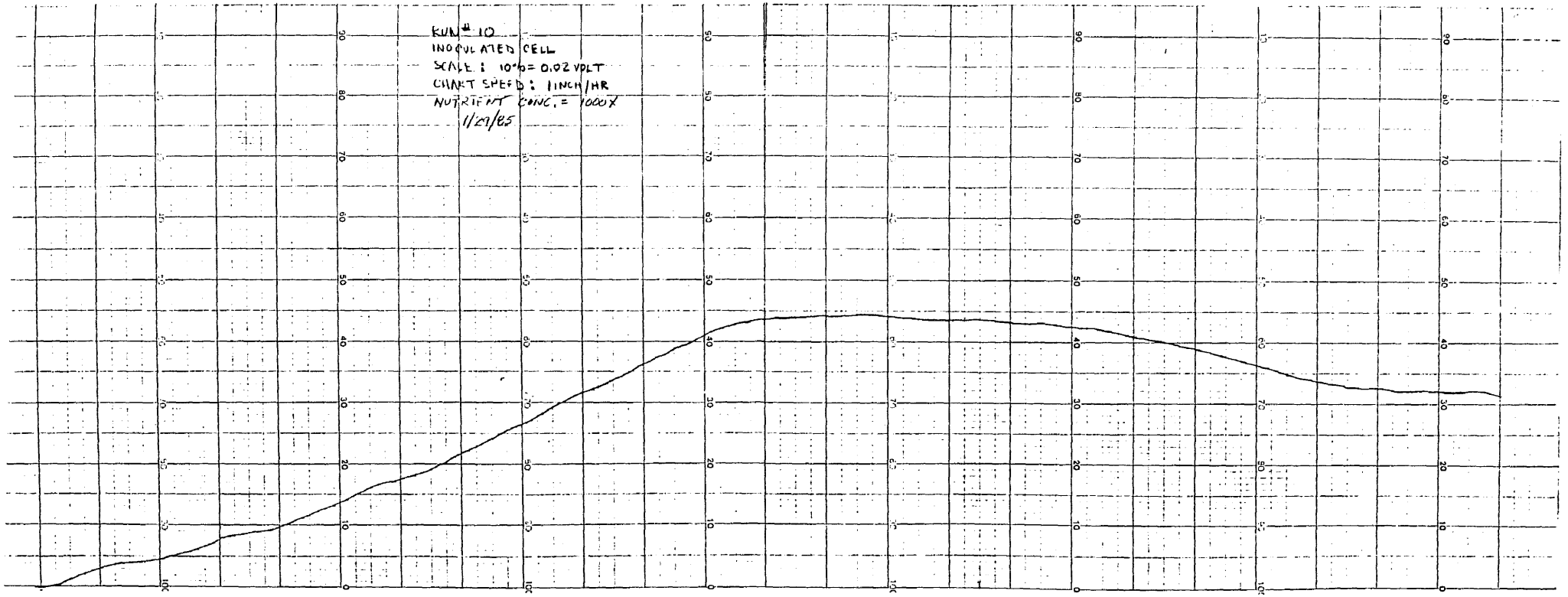


CHART 44
INOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

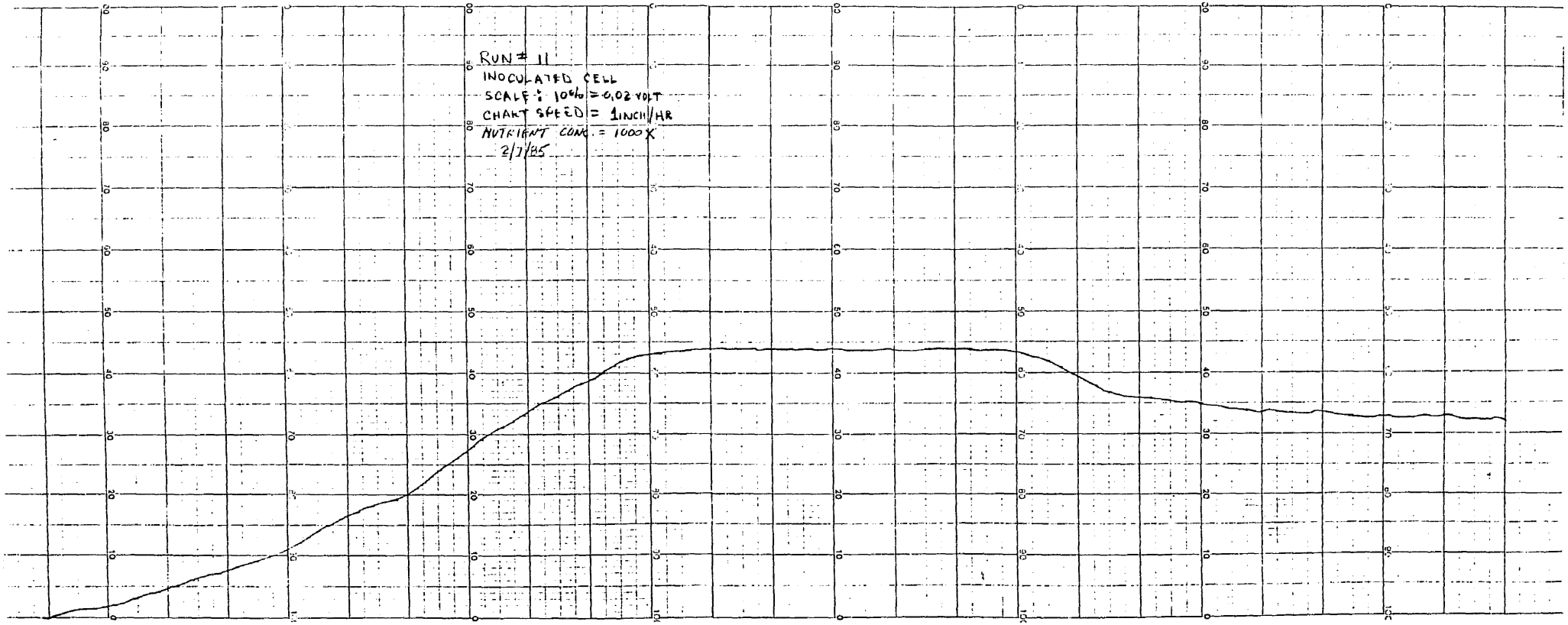


CHART 45
INOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

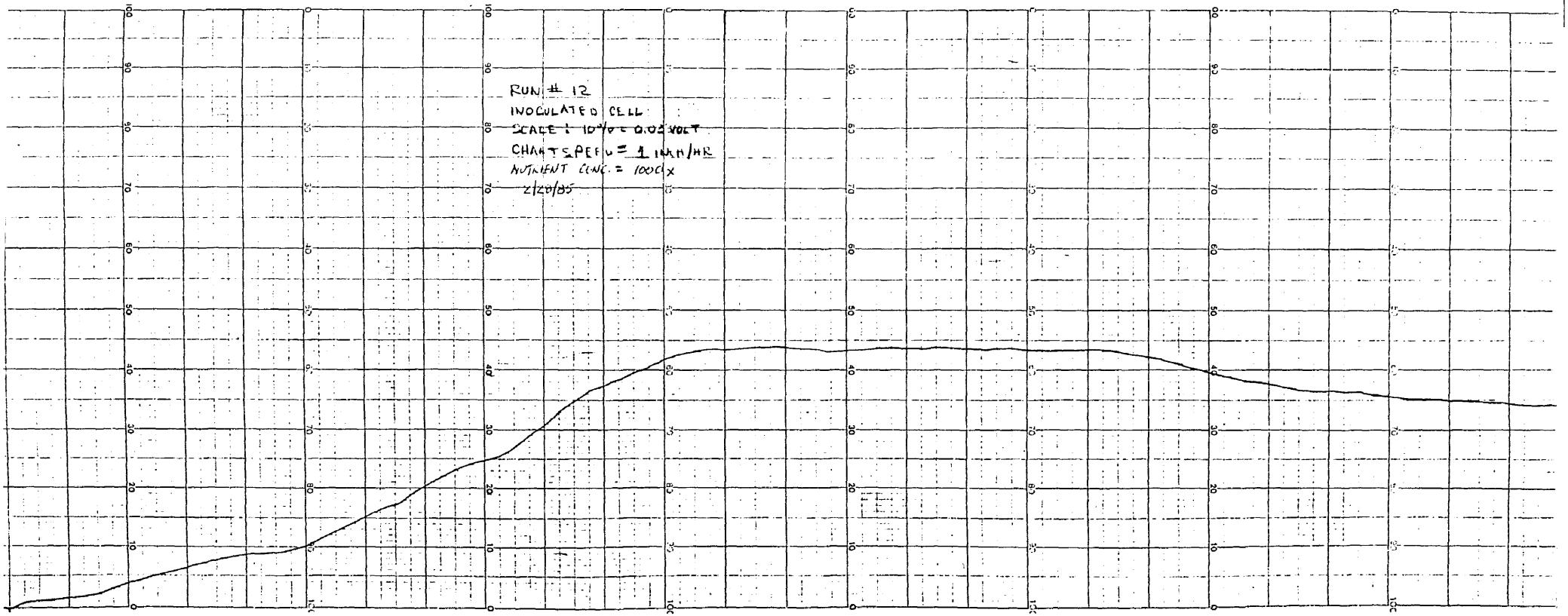


CHART 46
INOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

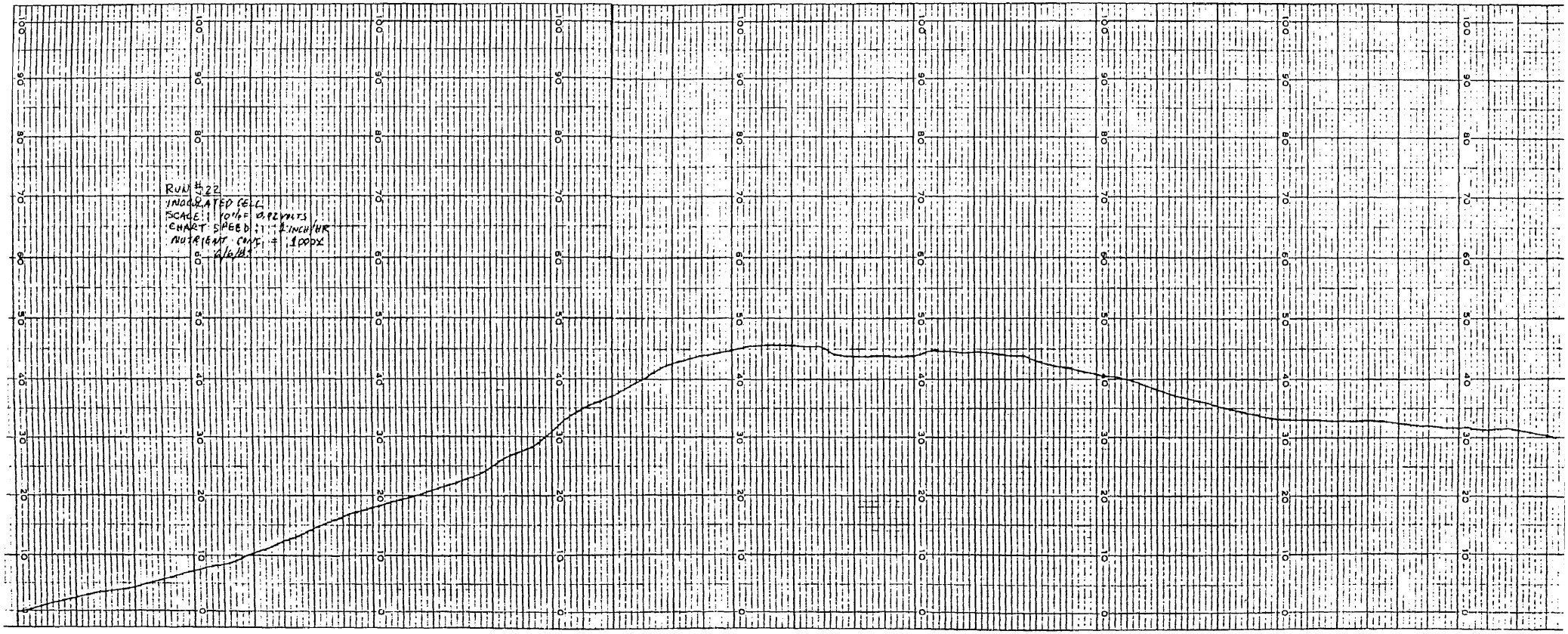


CHART 47
INOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

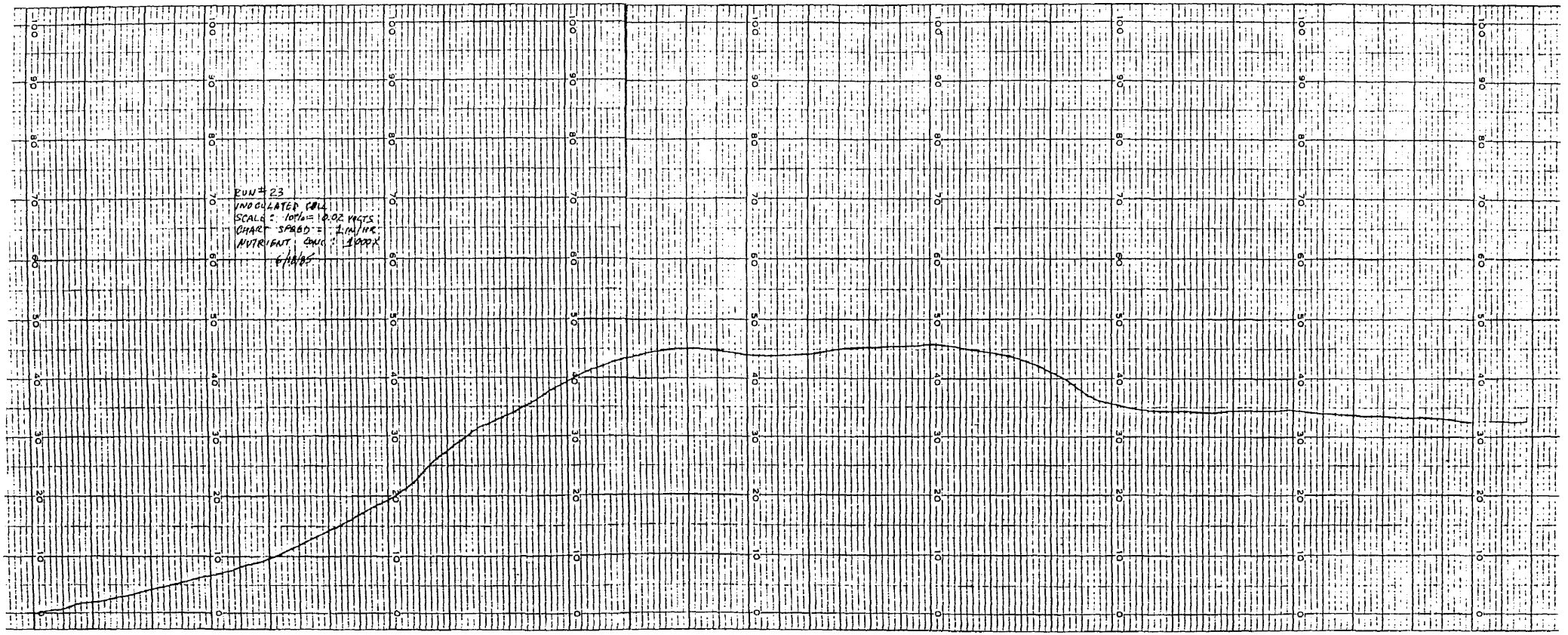


CHART 48
INOCULATED FUEL CELL OUTPUT
1000 FOLD CONC.

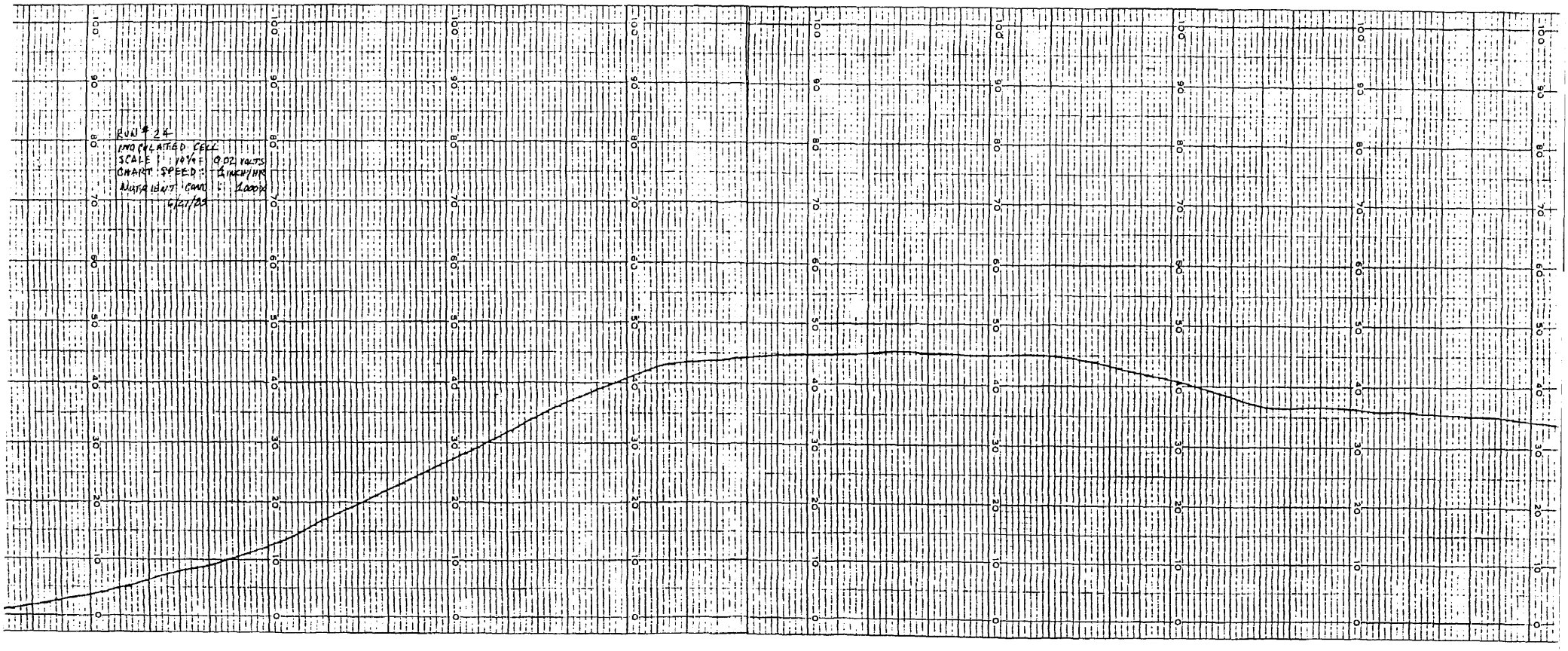


CHART 49
ACTIVATED SLUDGE FUEL CELL OUTPUT
INITIAL CONC.

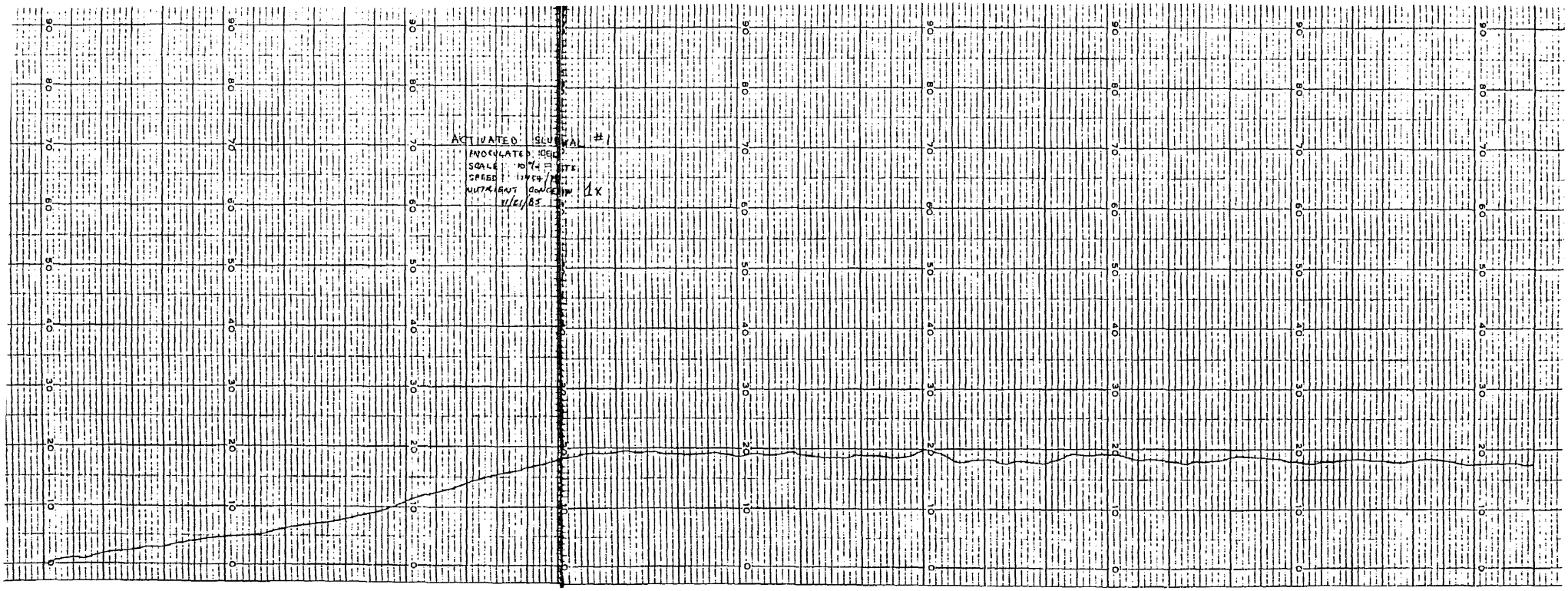


CHART 50
ACTIVATED SLUDGE FUEL CELL OUTPUT
INITIAL CONC.

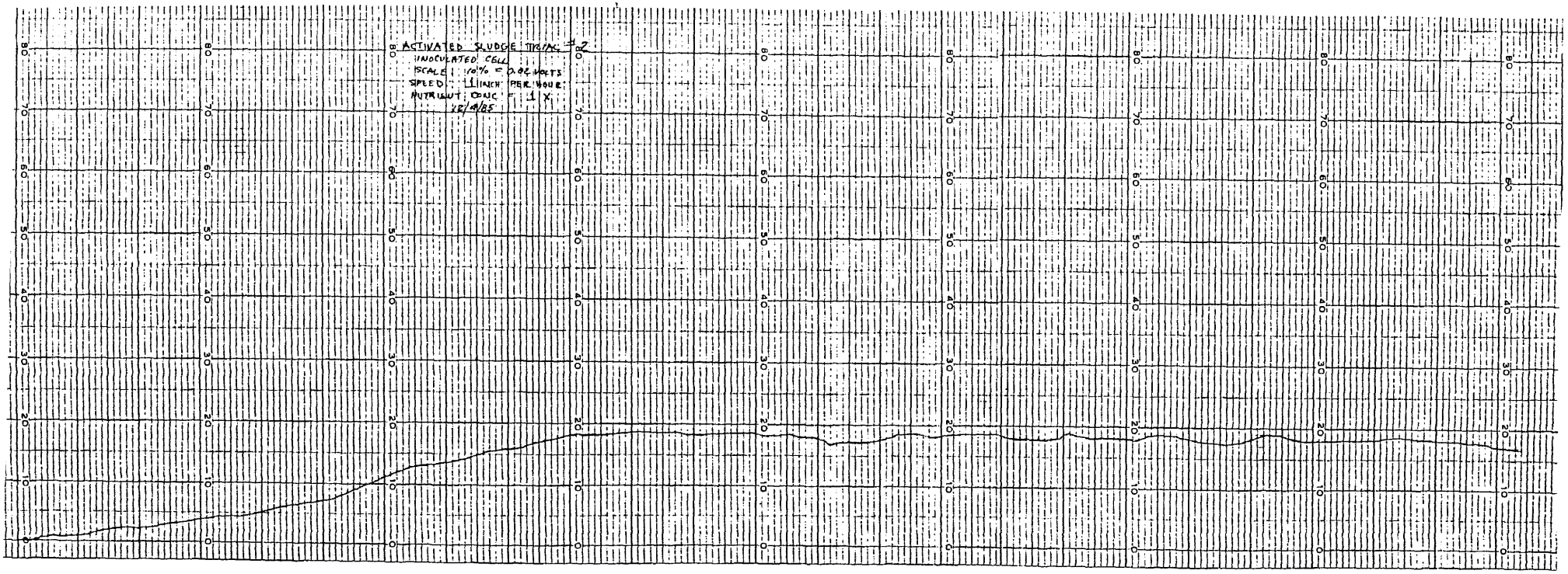


CHART 51
ACTIVATED SLUDGE FUEL CELL OUTPUT
INITIAL CONC.

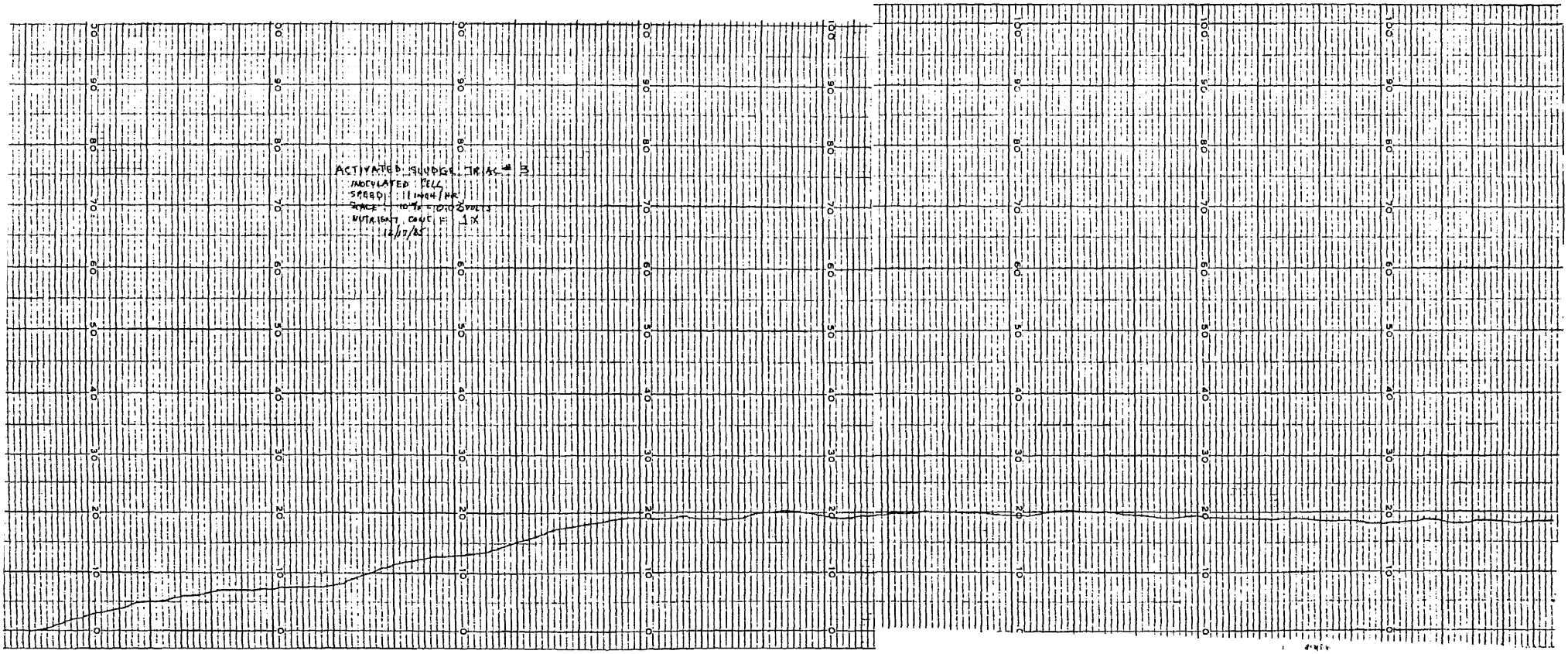


CHART 52
ACTIVATED SLUDGE FUEL CELL OUTPUT
INITIAL CONC.

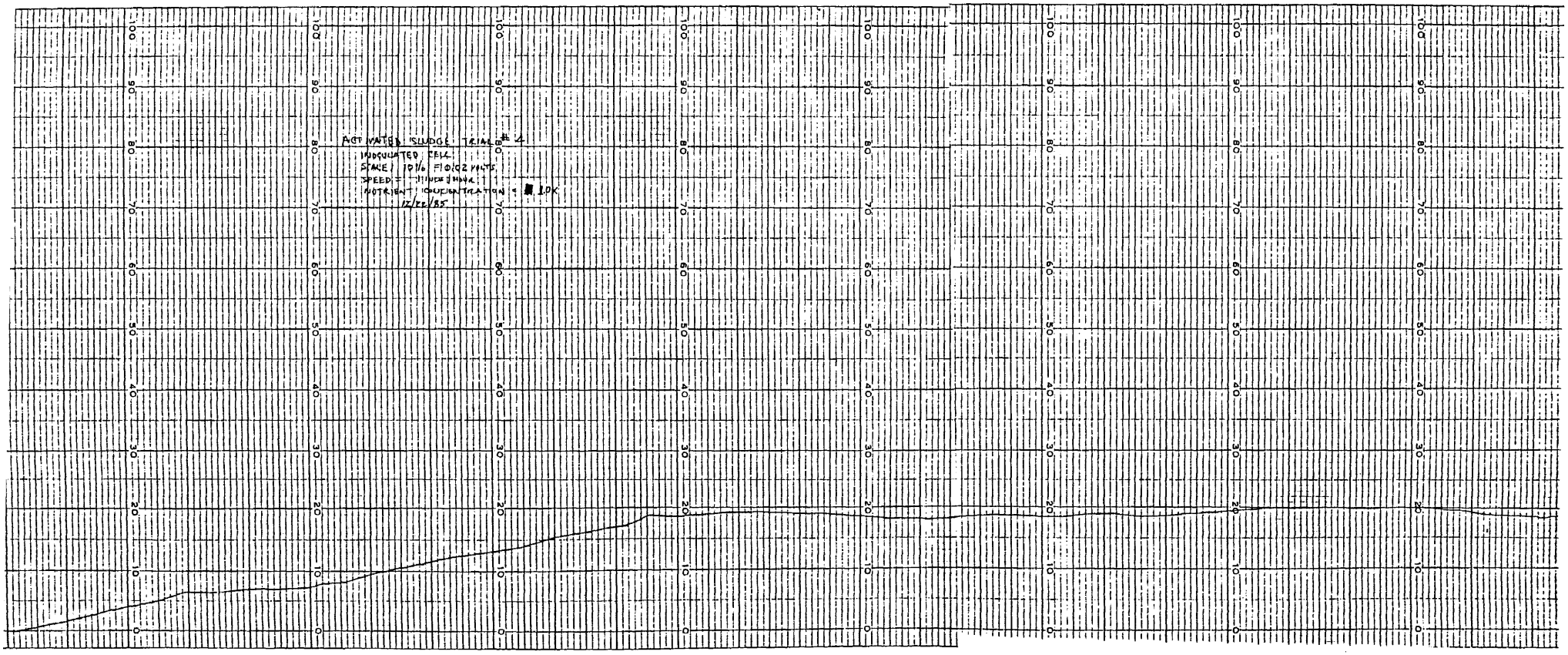


CHART 53
ACTIVATED SLUDGE FUEL CELL OUTPUT
INITIAL CONC.

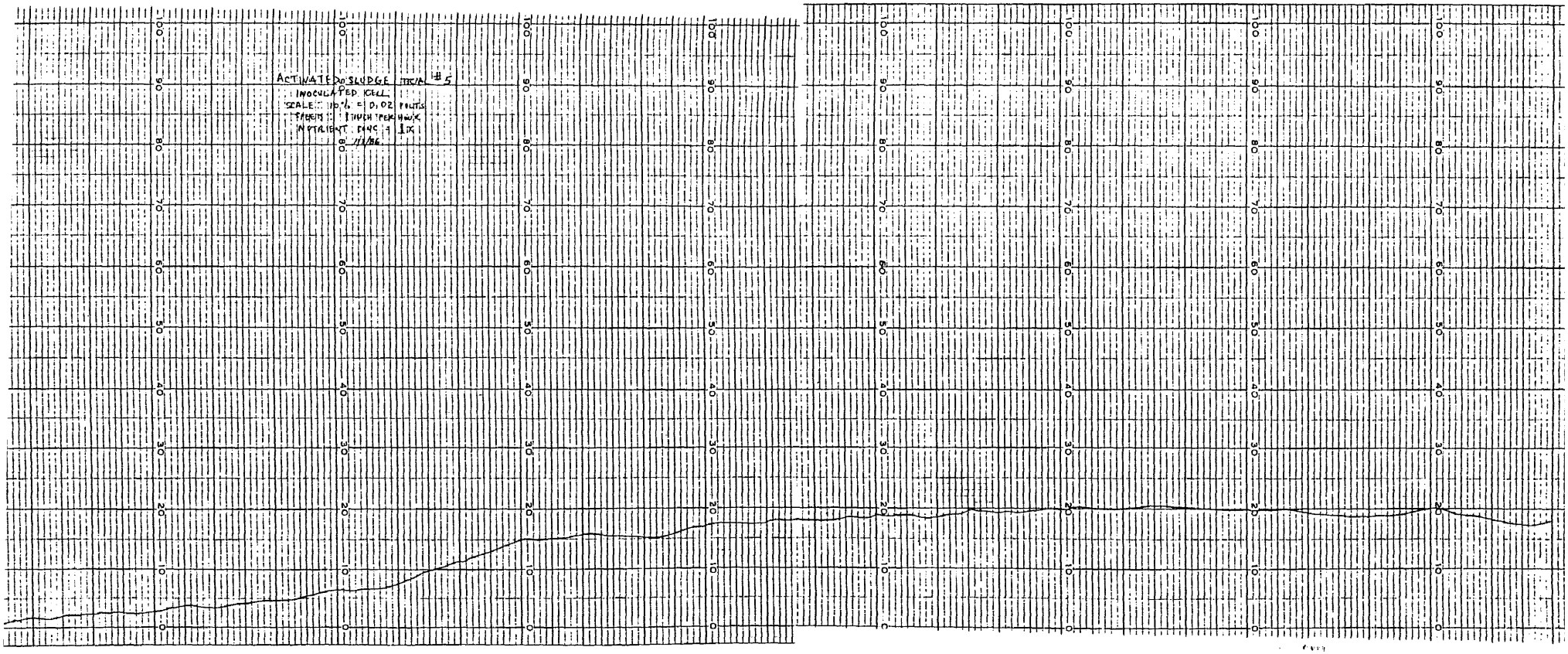
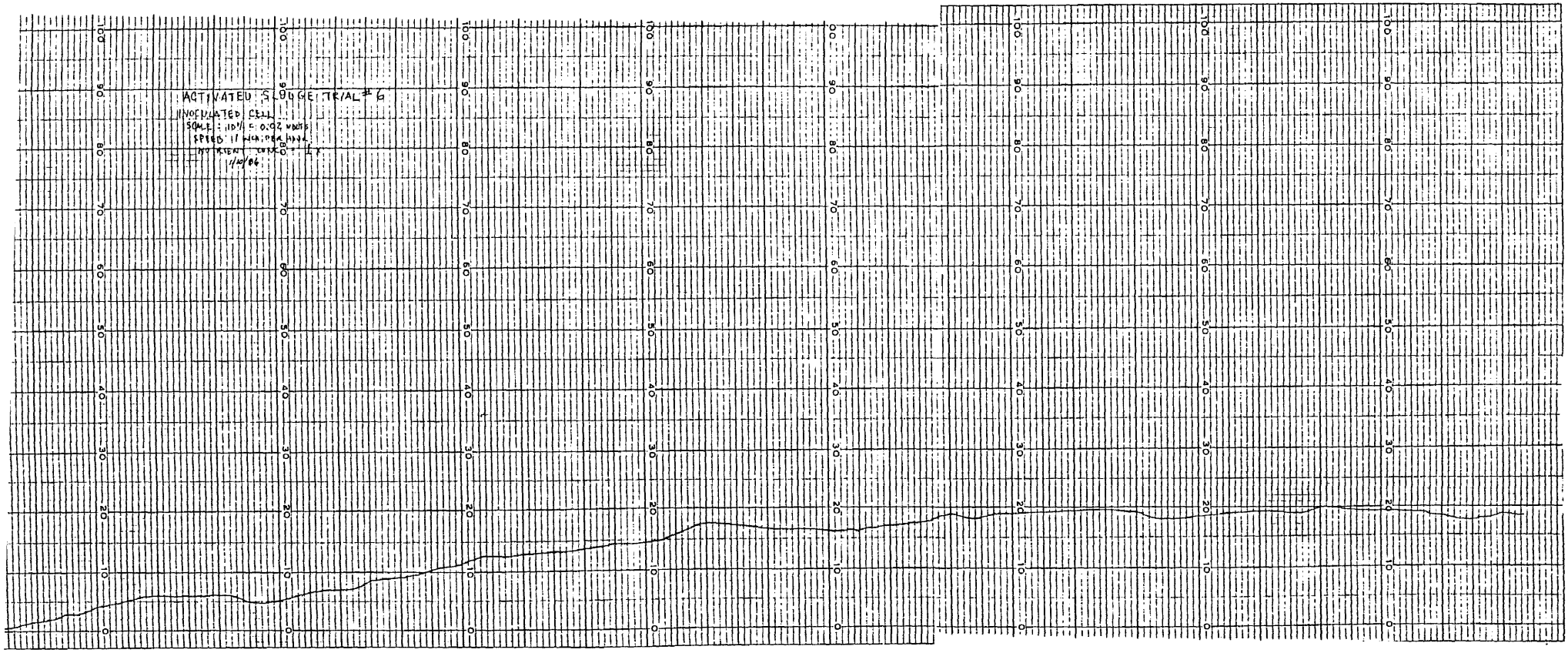


CHART 54
ACTIVATED SLUDGE FUEL CELL OUTPUT
INITIAL CONC.



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