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Development of an Automated Continuous Algal Culture Production System

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ABSTRACT

A continuous algal culture system consisting of three growth chambers, a nutrient feeding system, disinfection system, lighting and air/CO_2 system, and monitoring block is controlled by a Kaypro 2X micro-computer interfaced with a Remote Measurement Systems ADC-1 data and acquisition control board. BSR Type X-10 control modules manage additions to and withdrawals from the system. The TURBO Pascal control program is a user friendly, menu driven program providing for automated control of all routine operations associated with algae production. This design reduces the time and space input currently associated with algae production.

Chlorella sp. was chosen as the test species and has been maintained in dominance in the chambers for the past seven months. Average production levels of 135 g/m^3/day (dry weight) were achieved. Operational difficulties included occasional transformer failure, loss of positive system pressure, and minor communication problems with the BSR control system. This continuous algal culture system has proven reliable on an experimental level and commercial implementation appears feasible.

INTRODUCTION

Captive rearing of commercially important fish, molluscs, and crustaceans is an expanding industry, one that will have to continue to expand to compete with world-wide food markets. However, those facilities utilizing microalgae as the primary postlarvae food source are limited in their expansion capabilities due to the inability to economically scale-up algae production systems (De Pauw et al., 1983; De Pauw et al., 1984; Walsh et al., 1987; James et al., 1988; Guillard, 1975).

Traditionally, a multi-step backup batch system has been utilized in hatcheries where small quantities of algae are needed for short periods of time (Droop, 1975; Guillard, 1975; James et al., 1988; De Pauw et al., 1984). In this type of system, an axenic stock culture is used to inoculate cultures of increasing volume until the desired volume is reached. While this method successfully produces algae, it is not cost-effective due to the time, space, and hours required to maintain high production levels. In addition, the dynamic nature of batch cultures may result in nonuniform cell composition of the harvested algae.

Nurseries, on the other hand, require mass quantities of algae, thus, prohibiting indoor batch cultures as a cost-effective method of algae production. Therefore, nursery operators find themselves relying on natural phytoplankton present in the seawater, induced blooms of naturally occurring phytoplankton, or outdoor cultivation ponds for large-scale algae production. Harvesting naturally occurring phytoplankton is a rather sporadic method of obtaining food since production is greatly influenced by environmental conditions. In addition, control of the species present at any one time is difficult (Walsh et al., 1987; De Pauw et al., 1983). Use of large-scale induced algal blooms has seen limited success on the Belgian

coast (De Pauw et al., 1983). The drawbacks of this method include early collapse due to prevailing environmental conditions and lack of species control (De Pauw et al., 1983; Goldman, 1979). Outdoor cultivation ponds tend to be successful for abbreviated periods of time and then suffer the same fate induced natural algal blooms do. Walsh et al., (1987) have developed a continuous flow algal pond that is successful for certain times of the year. Failure occurs when light and temperature change drastically. In addition to short term productivity success, outdoor cultures require tremendous time and space.

Continuous culture techniques are used, but mainly on the experimental and small-scale level. Various cases of successful small-scale continuous cultures have been reported (Palmer et al., 1975; Trotta, 1981; Ukeles, 1976). James et al., (1988) recently reported average daily production rates of 117 g/m^3/day for Chlorella MFD-1 in continuous cultures. These systems show considerable production increases over batch culture techniques, but scale-up problems still exist due to manual operation practices.

Introduction of computer automation greatly reduces operating costs and labor intensity. This paper presents the design of an automated continuous culture system capable of maintaining species dominance over extended time periods, describes the control program, presents preliminary production results, and provides recommendations for future work and adaptation to commercial sectors.

BACKGROUND

The theoretical basis of continuous cultures (chemostats and turbidostats) was first developed by Monod (1950). He studied the relationship between substrate concentration, dilution rate, and specific

growth rate and developed an empirical equation describing this relationship. Herbert et al., (1956) validated Monod's theory with quantitative data, while Powell (1955, 1956) expanded it by looking at the relationship between growth rate and percentage occurrence of different aged cells and different generation times in continuous cultures.

A chemostat, simply a controlled environment or a fixed-volume chamber, allows an algae culture to grow exponentially in a well-mixed flowing medium for an extended time period. This system is characterized by the addition of fresh media at the same rate as the removal of algal cells, metabolic products, and used media. By finding the rate of inflow of media that equals the nutrient utilization rate of the algae, cost-efficient use of the nutrient supply can be achieved.

Turbidostats have also been utilized for producing algae. A turbidostat is a specialized chemostat, differing only in the method of culture maintenance. The density is measured by a photocell which controls the media flow rate (Ricica, 1958). In this manner, the flow rate is not constant but varies with the optical density of the culture. This system is essentially operated in the semi-continuous mode, in contrast to a continuously operated chemostat.

Chemostats and turbidostats are advantageous over batch cultures in that the culture can be maintained in the selected growth stage for indefinite time periods. The continual input of media, in combination with constant mixing, produces a more homogeneous culture, thereby, making determination and, subsequent automation of operational parameters easier.

METHODS AND MATERIALS

Experimental Apparatus

The production system contains three growth chambers constructed of

fiberglass-reinforced polymers sheets (Solar Components Corporation) with a light transmissivity of 92 percent (Figure 1). Each chamber is 12 inches in diameter, four feet in length and has a volume of 23.5 gallons. The chamber tops are friction-fit caps, allowing for venting, while the bottom is made of reinforced fiberglass.

The nutrient feeding system consists of 2 two-liter media bottles, a 20 liter brine solution carboy, two fixed-rate peristaltic pumps (Cole-Parmer, Model No. N-7531-00), and an activated carbon column (0.28 ft^3 of active carbon) for tap water dechlorination. The disinfection system contains a tap water source, a 20-liter concentrated disinfectant carboy, and a fixed-rate peristaltic pump. Solutions from both of these components empty into a common manifold, are picked up by a 1/25 HP chemical solution pump (Little Giant, Model No. TE-3-MD-HC), and added to the chambers through a 3/4 inch opening in the bottom plate (Figure 1).

Figure 2 illustrates the schematic of the lighting and air/CO_2 systems. Illumination is provided by four banks of lights (40W cool white fluorescent), one placed vertically in front of each chamber and one bank placed horizontally across the three chambers in the back. Aeration is supplied to the chambers by a 1/4 HP Thomas air pump rated for continuous duty. Dispersion is accomplished with two low-pressure, fine bubble air diffusers (average pore size of 35 um) placed at the bottom of each chamber. CO_2 is injected into the airline from a pressurized cylinder. The air/CO_2 mixture is filtered through a 0.80/0.45 um pore size prefilter and then a 0.20 um pore size filter.

System Monitoring and Control

The individual components of the experimental system are interrelated and controlled by a Kaypro 2X micro-computer interfaced to monitoring

devices with a Remote Measurement Systems ADC-1 data acquisition and control unit (Remote Measurement Systems, Inc., 1983). The ADC-1 allows for 16 analog inputs, 6 controlled outputs, 4 digital inputs, 32 BSR units, and RS-232 communications. The temperature probes, solar cells, fluorometer, and any other precision measurement instruments are connected to analog input channels. The ADC-1 receives analog signals from measurement instruments, converts them to digital signals, and relays these to the computer through RS-232 lines. This information is monitored and used by the control program to make procedural decisions.

Constant volume is maintained in the chambers by level detectors (two pieces of stainless steel wire separated by 1/4 inch). One of the wires is connected to a controlled output channel while the other one is connected to an analog input channel. The output channel provides power to the cathode during monitoring periods creating a measurable voltage across the analog input which is affected by the closure of the probe by water contact. The water level detectors are only intermittently activated to avoid problems with anodic corrosion associated with the brackish water and direct current (5v) power sources.

Pumps and solenoid valves, used for additions to and withdrawals from the chambers, are controlled by BSR Type X-10 remote control units. These are standard control devices available at most electronic stores. The computer sends a signal to the ADC-1 transmitter which generates and sends a digital code superimposed over the AC wiring to the BSR control units. The pumps are plugged directly into the BSR units. The solenoid valves use 24 volt AC current, requiring a transformer between the BSR unit and the valve to step down the line voltage to 24 volts.

System control is accomplished through a user friendly, menu

driven program written in TURBO Pascal. Figure 3 illustrates the flow chart of the control program. The program contains a supervisor stack sequence controlling the daily routine operations of the system. The stack sequence is written for optimal control of Chlorella sp. and will change depending on the algal species being grown.

When the program is initiated, the user has a choice of either activating the supervisor or manually selecting various procedures by the use of toggle keys. The supervisor mode contains two processes. First, the supervisor watches the internal clock and the time associated with the command at the top of the stack. When the two times are the same, the supervisor calls that particular procedure associated with the command. The activated procedure executes instantaneous operations, adds delayed operations to the stack, and relinquishes control back to the stack supervisor procedure immediately. The stack is loaded through the stack sorting procedure which prioritizes execution chronologically. With this organization, each control block (lighting, monitoring, harvesting, disinfection, and nutrient feeding) can be programmed independently utilizing time of day execution and condition verification loops to avoid conflicts. For example, crude turbidity measurements inferred from the output of the photocells are checked prior to harvesting to preclude the harvesting of a recently disinfected chamber. If necessary, the operator can override the supervisor and perform processes by triggering the appropriate toggles.

Experimental System Operation

The experimental system is currently operated in a semi-continuous mode, similar to a turbidostat. Air, continuously supplied to the chambers, is injected with CO_2 every minute for five seconds by opening and

closing the solenoid valve on the pressurized cylinder. Before the mixture enters the chambers, it passes through two filters to eliminate possible contamination sources.

Daily routine processes include monitoring, chamber harvesting, and nutrient and brine solution additions. Relative algal densities are determined by a solar cell placed on the back side of each chamber. The amount of light coming through the chambers, detected by the solar cells and transmitted to the computer, determines the amount of algae harvested. Though light intensity readings are taken every hour, harvesting occurs only once a day. Algae is allowed to flow from the chamber for a time corresponding to the most recent light intensity reading. Upon harvest completion, the supervisor calls the nutrient feed procedure to refill the chambers with dechlorinated tap water, brine, and media. After a specified time, the computer sends an "off" signal to the BSR modules controlling the media and brine pumps while the water remains on until contact is made with the level detectors. The power to the level detector is again turned off to avoid corrosion. Weekly chamber disinfection, in addition to the daily routines, completes the control sequence.

Weekly chamber disinfection prevents growth of periphyton which may have been inadvertantly introduced to the system. By disinfecting only one chamber on a given day, normal harvest levels are maintained. When the disinfection procedure is called, the chamber is harvested, filled with disinfectant and tap water, and allowed to soak for 15 minutes. Aeration of the chamber is continued during disinfection, providing agitation, and subsequently, some scrubbing of the side walls. After disinfection, the chamber is rinsed three times with tap water to ensure complete removal of disinfectant. Following disinfection, the supervisor calls the equilibrium

procedure which reinoculates the disinfected chamber from one of the other two chambers. Finally, the nutrient feed procedure is again called to add fresh media to the chamber.

Algae Culture

A axenic culture of Chlorella sp. (number 580) was obtained from the University of Texas-Austin collection. The culture was started by inoculating 250 ml. erlenmeyer flasks containing Guillard's f/2 media and dechlorinated tap water. The use of tap water significantly reduced operational costs. After grow-out, these cultures were transferred to two-liter flasks which were used to inoculate the experimental chambers. After initial inoculation, the daily and weekly processes were controlled by the supervisor for the duration of the seven month testing period, except for short time periods for program modifications. Initial values for temperature, pH, salinity, and illumination were assumed and modified throughout the testing period. Temperature varied in the system with changes in the room temperature, and thus, was not directly controlled. pH, salinity, and illumination changes were made by modifying the control program.

Once an observed steady state condition was reached, a preliminary set of kinetic tests were performed to determine the production capabilities of the system. Since the system operates in a semi-continuous mode, samples were collected prior to and after each chamber was harvested to get time $t = 0$ and $t = 1$ data points. The samples were filtered through 0.45 um membrane filters and analyzed for total suspended solids (TSS) (APHA, 1985). The TSS values were used to calculate daily specific growth rates.

RESULTS

Chlorella sp. was maintained in dominance for a seven month period following initial chamber inoculation from the two-liter flasks. The culture was still producing quite well when experimental testing was voluntarily terminated to permit installation of a refined prototype. Table 1 presents the parameter ranges used during the experimental period. By maintaining pH, temperature, salinity, and illumination in the listed ranges and without CO_2 addition, average production levels of 135 $g/m^3/day$ were achieved. Further increases in production are anticipated as the operation parameters are optimized. pH values initially started at 7.2 and were maintained at or below 7.5 with CO_2 addition. After CO_2 addition was terminated, pH rose to 7.8. Even though a 0.6 pH unit change was observed, no physical differences in the algal culture were detected and harvesting rates remained constant.

Though direct temperature control was not possible with this system, only a small variance was seen. Salinity variation between 1 and 10 ppt. caused no observable difference in production levels. Technically, Chlorella sp. is a marine species but easily adapted to low salinity concentrations in the chamber.

Though the present system successfully maintained Chlorella sp. in dominance for a seven month period, some problems occurred that were either resolved during the testing period or will be addressed in the next prototype.

Problems were encountered with the friction-fit caps. First, complete utilization of the chambers was prohibited; therefore, the top six inches of the chambers were left empty. Aeration caused periodic foaming of the cultures leaving rings on the unfilled portion of the chambers. Since

Table 1. Parameter ranges utilized in the experimental system.

Parameter	Range
pH	7.2 - 7.8
Temperature (degrees Celcius)	21 - 22.5
Salinity (ppt)	0 - 10
Surface Illumination (lumen/m^2)	5400 - 5500

liquid levels in the chambers were controlled by detectors, the top six inches were never disinfected and the rings built up after extended chamber use. This did not cause any functional problems, but was not aesthetically pleasing. In addition, the air pressure forced the friction caps open, allowing unfiltered air to freely enter the chambers and resulting in at least one case of contamination of the culture with an undesirable species.

Secondly, the transformers (Radio Shack, Model No. 66LEIB - 001) utilized to decrease the line voltage from 110 volts to 24 volts occasionally experienced burnout, preventing proper operation of the selonoid valves. Clearly, a more reliable commercial grade transformer must be utilized. Thirdly, three instances occurred where line noise and/or power surges caused by crowding of the outlets sent misqued signals to the BSP units, resulting in harvesting or disinfecting at inopportune times.

Fourth, only two banks of lights initially provided illumination for the entire system. During this time, a diatom, Nitzschia, started to grow and compete with Chlorella sp. for nutrients. Nitzschia was undoubtedly introduced to the system during the initial shakedown period as a result of the pressure cap failure. Two additional banks of lights were added, producing a surface illumination of 5400 - 5500 lumen/m^2 and creating an environment in which Chlorella sp. kinetically dominated Nitzschia. The resulting culture contained a contamination level of Nitzschia of approximately three percent (by cell count).

DISCUSSION

Maintaining Chlorella sp. cultures in dominance for extended time periods was demonstrated during a seven month testing period. The observed production levels compare well with other continuous methods in use today.

Laing and Jones (1983) reported production levels of 130 - 187 g/m^3/day of Isochrysis using a turbidostat, while James et al., (1988) reported average production values of 117 g/m^3/day for Chlorella MFD-1. Though production levels are comparable, operational costs of this system are most likely lower due to its automation.

The fact that productivity remained the same with and without CO_2 addition could be a possible mode of further reduction in operating cost. There were no physical differences; however, nutritional differences will have to be looked at before assuming CO_2 addition is not necessary. Utilization of dechlorinated tap water instead of distilled water helped reduce expenditures. Although problems associated with using tap water did not appear here, source water could impact production in other localities.

This system assures species dominance through three mechanisms. First, the system is maintained under positive pressure by filtered air which minimizes the potential for contamination by undesirable algal species. Secondly, periphytic algal survival in the system is controlled by the disinfection loop. And, finally, environmental conditions were selected to assure that the Chlorella sp. dominated the phytoplanktonic niche.

The successful operation over a seven month period indicates the potential of large-scale applications. Present constraints on up-scaling the system include chamber expansion (volume and number) and optimization of the control program for other algae species.

CONCLUSIONS

The automated continuous algal culture production system presented in this paper has maintained Chlorella sp. in dominance for an extended time period, thus fulfilling the research goal. First, the threefold control

strategy appears practical. Secondly, Chlorella sp. production is robust in a continuous mode. Lastly, high production rates can be obtained with this approach.

RECOMMENDATIONS

Recommendations for future work include:

1) Design refinements need to focus on the elimination of the problems encountered with the first system. This will include a different chamber design to eliminate the friction-fit caps, selection of more reliable transformers, and implementation of additional verification loops to detect false signals to the BSR units.

2) Simplification of the entire system to improve the economics of algal production and to make commercial implementation as practical as possible.

3) Once refinement of the control program for Chlorella sp. is completed, tests with other algal species need to be performed.

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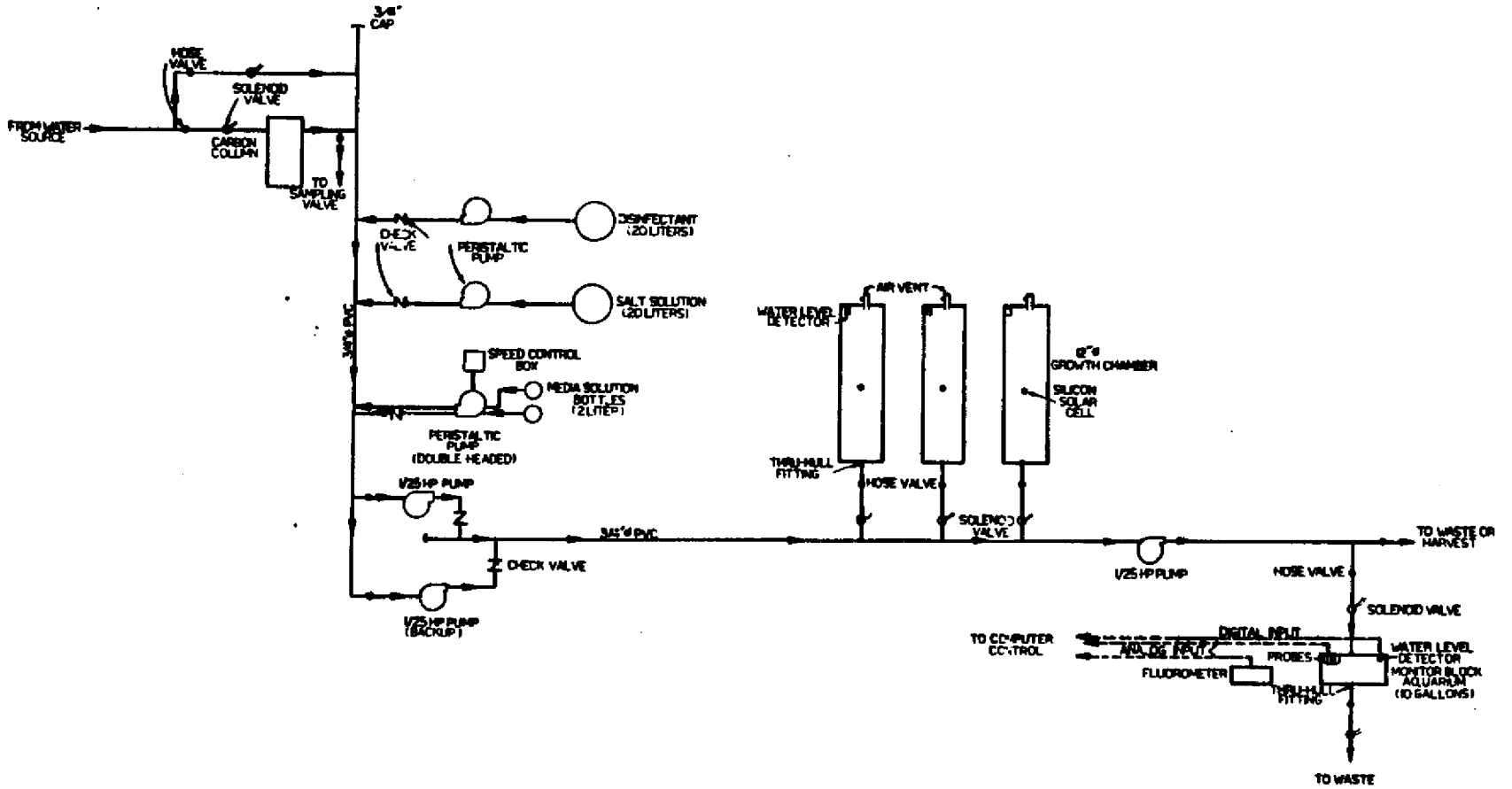

Figure 1. Layout of the nutrient, disinfection, monitoring, and computer control components for the continuous algal culture system.

NUTRIENT AND DISINFECTANT SYSTEM	
DATE: JUNE 14, 1988	FOR: KELLY RUSCH
APPROVED BY:	DRAWN: D.RADAICH

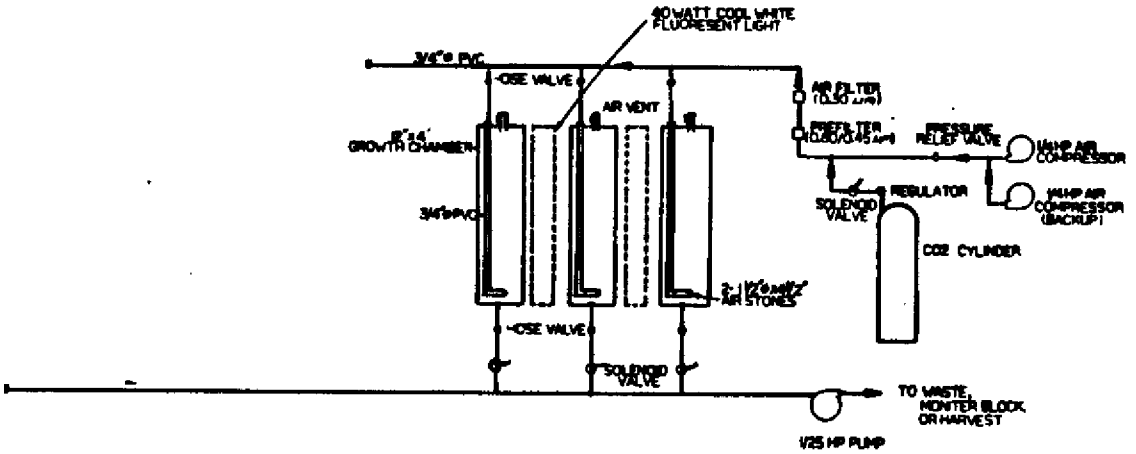

Figure 2. Layout of the air/CO_2 and lighting components of the continuous algal culture system.

AIR AND CO2 SYSTEM	
DATE: JUNE 15, 1988	FOR: KELLY RUSCH
APPROVED BY:	DRAWN: D. RADAICH

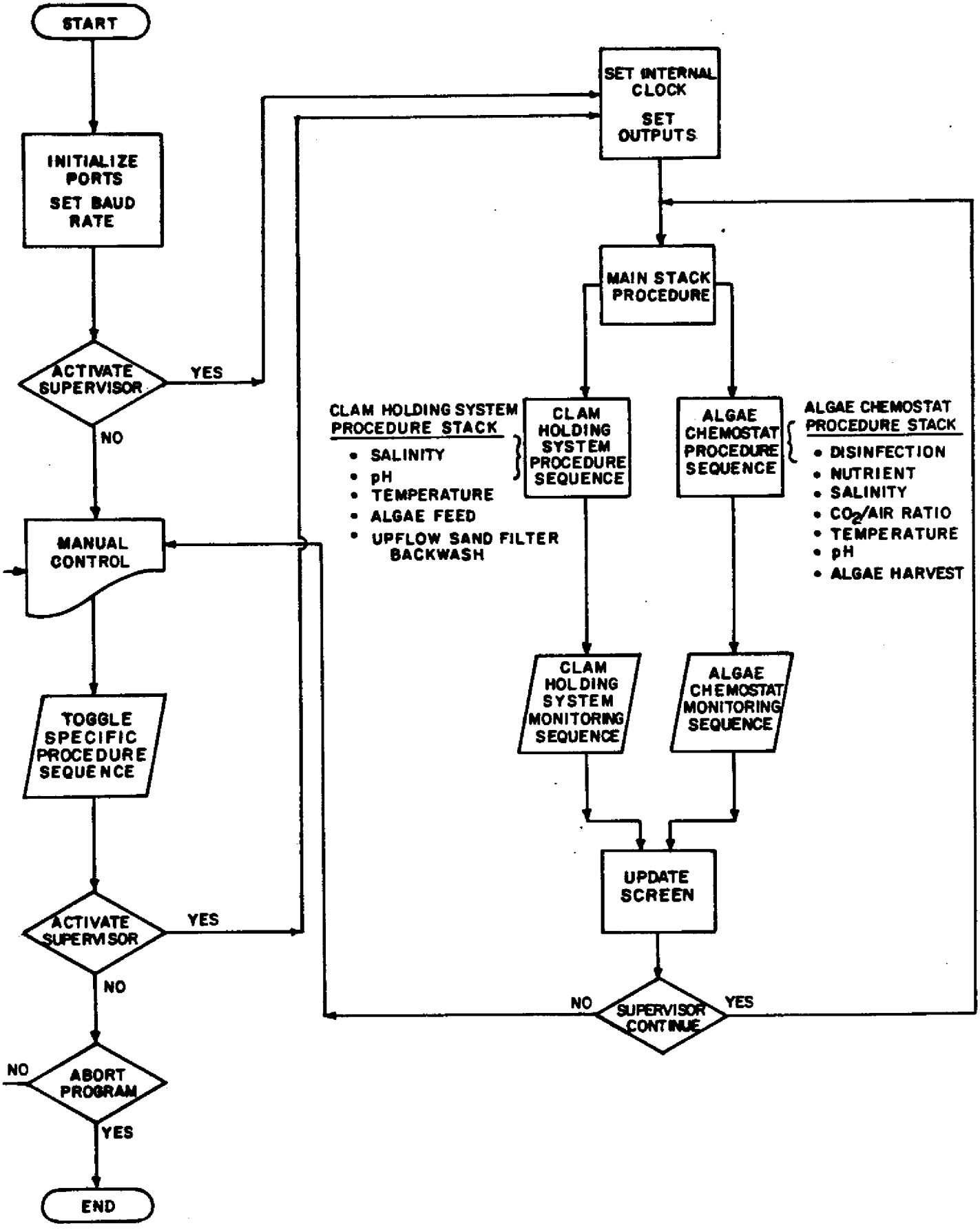

Figure 3. Flowchart of the control program for the continuous algal culture system.