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BACTERIAL DEPURATION BY THE AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*) UNDER CONTROLLED CONDITIONS

VOLUME II

PRACTICAL CONSIDERATIONS AND PLANT DESIGN

by

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Principal Investigators for this project were Dexter S. Haven and Frank O. Perkins. Both small scale and commercial scale depuration experiments were designed and carried out by Reinaldo Morales-Alamo and Dexter S. Haven. Special bacteriological studies and monitoring of the depuration experiments were conducted by Martha W. Rhodes and Frank O. Perkins. The engineering design of depuration ta-ks and plants was prepared by Bruce Neilson.

The findings and conclusions are presented in two separate Volumes:

Volume I. Biological and Technical Studies.

Volume II. Practical Considerations and Plant Design.

Special thanks must be given to Santo Furfari, project officer from FDA, who provided invaluable assistance and guidance. The advice and suggestions of the following persons also are gratefully acknowledged: Daniel Hunt, Assistant Chief, Shellfish Sanitation Branch; Albert Story, Chief, Gulf Coast Technical Services Unit; and Maynard Presnell, Deputy Chief, Gulf Coast Technical Services Unit.

INTRODUCTION

Shellfish are filter feeders and pump large volumes of water when they are active. Thus, shellfish may accumulate bacteria or other contaminants, should they be present in the surrounding water. This effect is sometimes called bio-concentration. The reverse process, called depuration, is the natural process of purification by which the shellfish cleanse themselves. In depuration, shellfish are placed in "clean" water and the accumulated pollutants are eliminated from the organism by mechanisms which are not well-known at present. This report is a guide for the depuration of bacteria from oysters in the waters of the Chesapeake Bay system. In general, the methods and principles described in this report will hold true for other shellfish, other contaminants and other estuaries, but the specifics and details could vary significantly.

Depuration has become a necessity in many areas because the number of persons living within the coastal zone has increased rapidly. The wastewaters from concentrated population centers have degraded the water quality in many nearby estuaries and bays. As a result, increasing acreages of the best oyster growing bottoms in the United States are classified as restricted for shellfish culture. In order for these shellfish to be used, they first must be cleaned or caused to cleanse themselves of bacteria and other particulate materials.

The laws relating to the use of shellfish from restricted areas vary from state to state, but most allow sale of the shellfish if they are relaid into approved waters for a specified period of time. For example, in Virginia, oysters relaid into approved waters may be harvested after 15 days when water temperatures are above 50°F. But relaying oysters is an expensive process which is seldom practiced since the cost of harvest is doubled; also, it is seldom possible to reharvest more than 80% of the crop. Therefore, depuration under controlled conditions offers an attractive alternative to relaying in natural waters. This process of controlled purification is what is usually meant by the term depuration.

The basic units of a depuration plant are neither numerous nor complicated. In most instances water will be taken from a nearby estuary, disinfected by ultraviolet light, flowed through the depuration tank and then returned to the estuary, as shown in Figure 1. Similarly, shellfish will be taken from nearby growing areas, washed and culled and placed in the depuration tank for a prescribed period of time. Once the purification has been achieved, the oysters will be sent to market. Modifications of this plan exist and will be discussed in later sections.

Studies conducted at several locations in the United States have shown that oysters, hard clams and soft clams may be freed from unacceptable levels of fecal coliform bacteria in about 48 hours, providing that certain conditions are met. Today, there are operational depuration plants in Maine, Massachusetts, New York, New Jersey and Delaware. These states have both state and federal regulations which permit and regulate depuration. In other states, regulations relating to controlled depuration have not been formulated.

Many of the bacteria that are removed from the shellfish are not necessarily harmful to humans. Rather they are "indicator organisms" which can be related to the presence of fecal pollution and the likelihood that pathogenic or disease producing organisms are also present. The organisms which are measured are the coliform bacteria. The precise methods followed for determining the presence and number of these bacteria can be found in Standard Methods (APHA, 1976). The tests can differentiate between the entire coliform group and those which have origin in the feces of warm-blooded animals, the so called fecal coliforms. Federal and state agencies have set standards for specific uses (eg. recreation, shellfish culture, drinking water supply and human consumption for shellfish). The trend in recent years has been away from the exclusive use of the total coliform count. The studies conducted included both measures, but for the sake of brevity and clarity only fecal coliform counts will be presented in this report. Persons interested in total coliform data should consult Volume I of this report.

The measure of coliforms is the number of organisms per unit volume for liquids and per unit weight for solids. In fact, precise determination of the number of bacteria is impossible and the numbers represent statistical estimates. The unit used is the MPN or Most Probable Number. The unit of volume for water samples is 100 milliliters (100 ml) and the unit of weight for oysters is 100 grams. In order to avoid unnecessary repetition, the

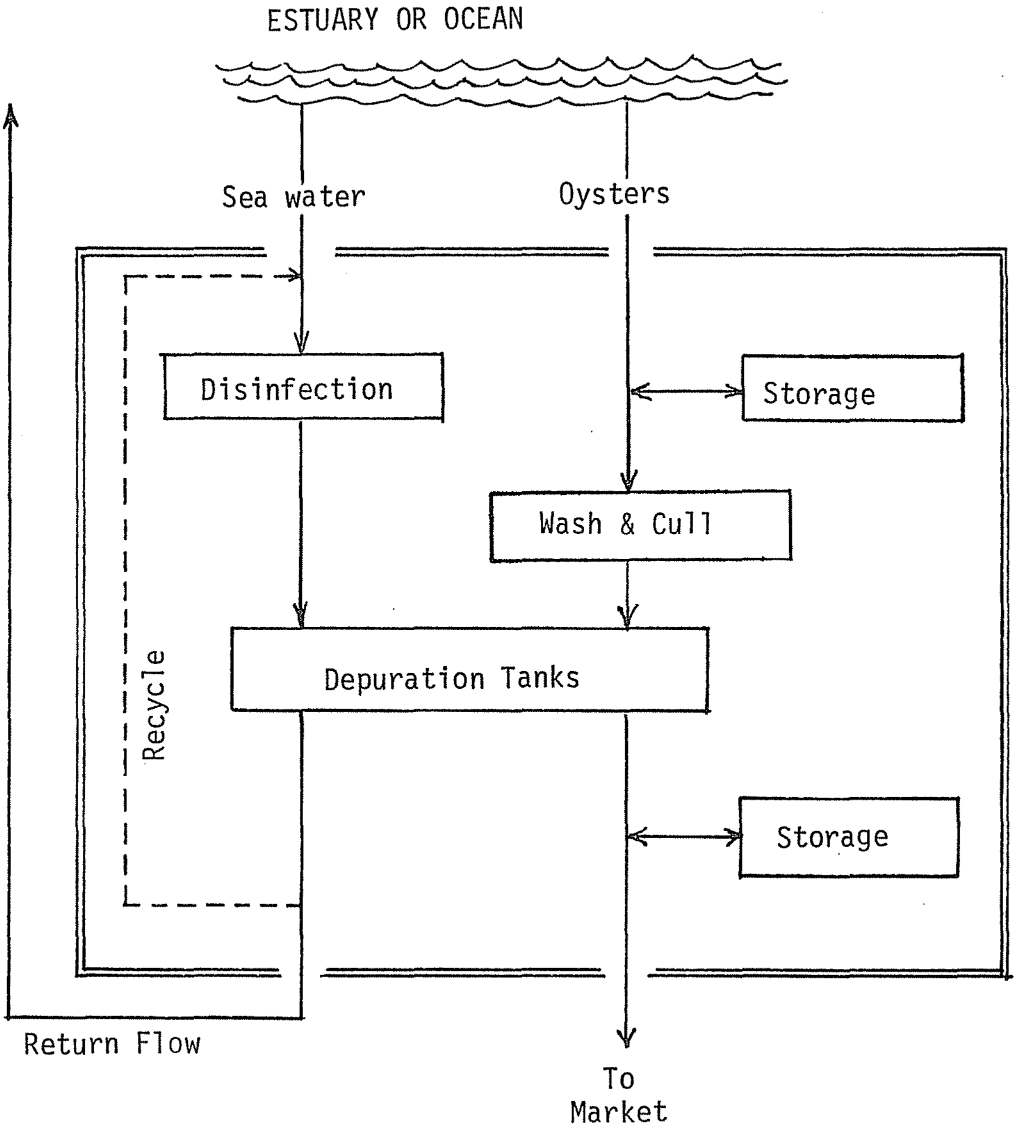


Figure 1. Flow of water and oysters through a depuration plant.

notation in this report has often been simplified. Thus, for a water quality discussion the statement that "25 coliforms were present" in fact means the most probably number of fecal coliforms in 100 milliliters of water was 25. Similarly, coliform counts for oysters will always be MPN of fecal coliforms per 100 grams, unless otherwise noted. Additionally, numerical values for bacteria represent geometric means of several samples, unless otherwise noted.

Depuration in general refers to the cleansing or purification process without specification of initial or final conditions. However, some standard(s) will be necessary to judge the success or failure of the depuration process; the criteria will be set by federal and/or state agencies. For the purpose of these studies, the existing federal standard for the allowable number of fecal coliforms in hard clams in the interstate market (namely MPN of 50 fecal coliforms per 100 grams) has been selected to determine the success of individual tests. This does not constitute a recommendation or an endorsement of this standard, but rather simply indicates the need of fixed reference points when evaluating test data.

Unless otherwise noted, mean values for physical and chemical environmental factors given throughout this report are based on measurements made over a three-day period. For example, the value of 77 mg/l given for turbidity represents the mean of a series of individual measurements over a three-day period and not an absolute upper limit.

Following sections contain general information concerning depuration plants and biological factors which are important. Later chapters include designs for the water supply system, the depuration tanks and other engineering aspects of the depuration plant, as well as suggested layouts for both a small and a large depuration plant. The final chapter deals with operating and monitoring procedures which are needed to provide public health safeguards.

This guide is based on data and conclusions presented in Volume I of this report. It includes a more detailed presentation of the depuration studies, discussion of the various environmental factors which affect depuration, review of rapid techniques for determining fecal coliform levels and other topics. It also includes the data on which the analyses are based. The reader who desires information beyond that presented in this volume is referred to Volume I of this report.

GENERAL CONSIDERATIONS

Very few definitive statements can be made regarding the siting, size and design of a depuration plant since local conditions must be accommodated. However, since both shellfish and seawater are required, economics dictate that a plant probably will be located on the water or only a few hundred yards away. Since most plants that are in existence today are located near the water, "open" seawater systems are used. That is, the seawater flows through the plant and is returned to the estuary after being used only once. Another name for this type of system is a "once through" design. A completely closed system with continuous recycling of the seawater is theoretically possible, but the potential for build up of undesirable water constituents must be considered. A semi-closed system could be both practical and economical if special treatment of the seawater (eg. heating) is required. In this instance, a sump or storage tank would be used. "New" water would flow into this tank at a slow rate, and an equal flow of "used" water would be returned to the estuary. Within the plant, water would flow through a treatment system and into the depuration tanks at a much higher flow rate. In this fashion water would be recycled frequently but during the period of a day, the water in the system would be replaced several times. This design might be economically attractive if depuration were practiced during winter months when the water temperature in the estuary was below 14°C (57°F). Biological considerations dictate that the water in the depuration tank be above this temperature, so that heating would be required. Heating expenses would be reduced if the warmer water were recycled before discharge to the river.

All but one of the tests presented in this report were once-through systems. In most applications this method would be the simplest and cheapest technique. In a few instances a semi-closed system with gradual renewal of the water could be both economical and practical. The authors do not foresee any situations in the near future which would require a completely closed or recirculating system. However, such an alternative is not to be completely ignored.

The size of the depuration plants is determined not by technical considerations, but rather by local conditions and economics. Factors which would be important are: the available supply of shellfish from nearby and more distant growing areas, the distance to markets, transportation expenses, rental fees for buildings and/or equipment, availability of part-time and full-time personnel. Certain fixed costs are incurred regardless of size. If a plant consistently processed small batches of oysters, the unit costs could make the plant unprofitable. For this reason and others, it has been estimated that a plant must process between 15 to 20 bushels of oysters per day to succeed. A large plant, on the other hand, might process hundreds of bushels per day. Details of the design for each case will be presented in later sections.

The indefinite nature of plant size need not cause problems. At present oyster culture and harvesting is not an automated or highly regimented industry, such as a factory or similar industrial processes. From a practical point of view, it is unlikely that any supplier could guarantee a precise quantity of oysters each day. When the work went well it would be foolish to abandon harvesting simply because a daily quota had been passed. Thus, some days an abundance of oysters might be available. On the other hand, weather conditions might preclude the harvesting of any oysters on other occasions. In short, the supply of oysters to the depuration plant is almost certainly going to be highly variable. Storage facilities to handle excess are required. To further accomodate this variability, a modular approach is recommended.

Even for small plants, it is recommended that a large number of small tanks (holding about 3 to 10 bu) be used rather than a small number of larger tanks. The small tanks will require somewhat more plumbing and be slightly more expensive, but a greater flexibility is given to the plant operator. If a small volume of oysters arrives on any given day, only the required number of tanks need be utilized, thereby conserving water and energy and minimizing expenses. If only large tanks are available, either the shellfish must be stored temporarily or else the tank must be used although only filled to a fraction of its capacity. Day to day variability can be handled quite easily, as can repairs, modifications and other variations when small tanks are employed. If these small tanks can be constructed from commonly available materials, such as standard size sheets of plywood, the cost of construction also will be reduced. These tanks would hold between 3 and 10 bushels of oysters each, depending on the particular design chosen.

The degree of automation probably will increase with plant size. In general, the oysters will be loaded into small trays (capacity of about one bushel) and a number of these trays placed in each depuration tank. This system is highly flexible, but is rather labor intensive. An alternative is to have larger combination tray-tanks that could be moved from one location to another by a forklift or similar mechanical device. In this manner the oysters could be loaded directly into the tray-tank, and the tray placed in a rack with the necessary plumbing for water supply and removal. Until detailed studies of the man-hours and equipment necessary for each system have been made, it is not possible to state the size necessary to make this method economical.

In addition to the primary units, namely the water system and the shellfish processing units, some support facilities will be needed. For example, refrigerated storage of oysters will be necessary. Some office space will be needed by the plant manager since detailed records likely will be kept for both public health and business purposes. At a minimum, adequate sanitary facilities for employees will be required, and if the number of employees is large, additional facilities such as lockers and showers may be desired and/or required by OSHA, or other regulatory authority. Storage for spare plumbing supplies, trays and other equipment will be needed, too. Laboratory space may be desired.

BIOLOGICAL CONSIDERATIONS

Although depuration is a natural process, man has a certain degree of control over it since he can modify the water environment within the depuration plant. The oyster's metabolic activity, especially the pumping rate, and other behavior patterns will vary in response to changes in the water environment. In order to achieve successful depuration, it is necessary to know how the environmental factors affect the depuration process. In general, we are most concerned about those variables which will alter the rate at which depuration will occur. Those factors which have been shown or may have the potential to have a significant impact are: temperature, dissolved oxygen content, salinity, phytoplankton concentration and turbidity.

Water Temperature

The temperature of the irradiated water entering the depuration tank should be within the range of 14° to $29^{\circ}C$ (57° to $84^{\circ}F$) if moderate to high levels of fecal coliform bacteria are to be depurated in a short period of time. Our studies indicate that, provided the other holding conditions are suitable, oysters cleansed themselves in 48 hours at these temperatures. The lower temperature may be extended to $10^{\circ}C$ ($50^{\circ}F$) provided the initial MPN levels of fecal coliforms in the oyster before depuration are below about 200/100 gm meat.

The temperature range is important in locating plants in the Chesapeake Bay region and other localities where seasonally the water temperatures may range from just below zero to over $30^{\circ}C$ ($86^{\circ}F$). The present study did not include experiments at water temperatures above $29^{\circ}C$ ($84^{\circ}F$). Therefore, we cannot make recommendations for temperatures above that level. However, it is known that the activity of oysters diminishes significantly at about $32^{\circ}C$ ($90^{\circ}F$). Furthermore, water temperatures above $30^{\circ}C$ ($86^{\circ}F$) encourage the rapid growth of bacteria in the depuration system.

Depuration is not recommended when water temperature is below $14^{\circ}C$ ($57^{\circ}C$), if natural unheated waters are used. Under these conditions, depuration should be attempted only from about mid-April to mid-November in the Chesapeake Bay system. Oysters may be depurated in the 10° to $14^{\circ}C$ range (50° to $57^{\circ}F$) if the initial fecal coliform level in the meats is 200 or less. In Chesapeake Bay during the warmer months, oysters from many restricted areas have fecal coliform counts exceeding 1000 per 100 grams of meats. However, as the temperature falls to below about 12°C, fecal coliform levels in oysters often decline (but not in all areas) to very low levels. Thus, depuration may be feasible in spite of the low biological activity of the oyster. Laboratory studies have shown that pumping, feeding and other activities of the oyster start to decline at temperatures below 12°C (54°F).

If depuration is to be practiced when estuarine water temperatures drop below 14°C (57°F), it is recommended that the water be heated if bacterial counts are higher than 200. Heat exchangers are available commercially and many designs exist which could be fabricated locally. It is recommended that the plant be operated as a semi-closed system to reduce heating costs.

Dissolved Oxygen

Oysters depurated in 48 hours at mean dissolved oxygen concentrations ranging from 0.8 mg/l to about 8.8 mg/l; at 0.6 mg/l, 72 hours of depuration was needed.

While this range appears wide, we do not recommend approaching the lower critical level, but recommend that the minimum value be no lower than 2 mg/l since our studies showed that depuration at oxygen concentrations above 1.8 mg/l was consistently satisfactory. Furthermore, if a plant were operated near the critical level, values might decrease to close to zero where water circulation was restricted.

While oxygen concentrations below certain limits are detrimental, there is also an upper limit determined by the solubility of oxygen in water which also could be harmful. The solubility of oxygen decreases with a rise in temperature and with an increase in salinity. A summary of the solubility of oxygen expressed in parts per million (ppm = mg/l) follows:

Saturation Values for Oxygen in Water
(in mg/l)

Salinity (ppt)	Temperature 10°C	20°C	30°C
10	10.7	10.1	9.4
20	8.6	8.1	7.6
30	7.2	6.7	6.4

When values exceed those shown above for any given temperature and salinity, the water is supersaturated with oxygen. When supersaturated water warms, it releases the excess oxygen (and other gases) in the form of bubbles, and these bubbles can cause death of oysters by embolism. Therefore, supersaturation of the water is to be avoided.

In flowing sea water systems, when the water is taken directly from an estuary and then flowed to a tank, dissolved oxygen is usually within acceptable limits (i.e. above 2 mg/l). However, when oysters are held in a recirculating system, the respiratory activity of the animals quickly depletes the available oxygen and it must be replenished by aeration. If the salt water comes from a well, it usually is deficient in oxygen and it must be oxygenated by aeration.

Levels of dissolved oxygen in sea water may be determined by analytical tests which are described in Standard Methods (APHA, 1976). We recommend, however, the use of an oxygen meter which may be purchased from several commercial sources. The desired levels of oxygen in the holding tanks may be achieved by a variety of aeration methods outlined later in this report.

Salinity

Oysters in nature grow and reproduce in regions where mean salinities range from about 5 ppt to 32 ppt (ppt = parts per thousand). Consequently, oysters may be depurated over a wide salinity range. Studies at Gloucester Point, Virginia, which is located on the estuarine portion of the York River, indicated successful depuration of fecal coliform bacteria with salinities ranging from about 14 ppt to 22 ppt. Studies in Alabama also indicated successful results over a wide salinity range and suggested 10 ppt as the lower level. Consequently, a range from 10 ppt to 32 ppt or over may be satisfactory for depuration if other aspects of water quality are within acceptable limits.

If oysters are moved from a high to low salinity or from a low to high, then a period of acclimation may be needed for resumption of normal pumping and feeding activity of the oyster. The time required will depend on the magnitude of the change and the water temperature at the time.

However, available evidence indicates that about 12 hours will be the maximum time, and in most instances, oysters will resume activity in a few hours. Oysters moved from an area having a water salinity of 4 ppt to an area with a salinity of 14-17 ppt (Gloucester Point) depurated fecal coliforms from very high levels in 24 hours.

While oysters may depurate over wide salinity limits and can tolerate sudden changes in salinity, it is recommended that depuration plants not be located in regions where mean salinity is lower than 10 ppt, since freshets would make depuration impossible at certain times of the year. Moreover, to avoid the acclimation time it is recommended that a depuration plant be located where the salinity is similar to that of the oyster growing areas. It is also possible to locate the plant near a source of high salinity water and use fresh water to dilute the seawater to the desired level if the fresh water meets standard criteria with respect to microbes and chemical quality. Residual chlorine in treated drinking water supplies could preclude use of that source.

Phytoplankton

There is no evidence that the quantity of algal food materials in the water influences the depuration process. There is, however, a major exception to this generalized statement. In Chesapeake Bay during the warmer months there sometimes occur dense "blooms" of dinoflagellates which may color surface waters in an estuary brown or red (often referred to as "red tide" or "red water"). When sufficiently dense, they inhibit oyster pumping activity and often settle to the bottom of the holding tanks where they quickly reduce dissolved oxygen content to low levels. There seems to be no region in Chesapeake Bay where this phenomenon has not occurred. We recommend that depuration be approached with caution during these dinoflagellate blooms when the estuarine water color is noticeably altered. A quantification of what constitutes a "bloom" is not possible at this time. The depuration plant operator should base the decision to suspend depuration operations on the coliform levels in his depurated oysters. If levels do not drop below the maximum accepted value, the operations should be stopped.

Turbidity or Total Suspended Solids

Oysters in Chesapeake Bay successfully live where natural turbidity is high. Oysters depurated when they were experimentally subjected to mean turbidity levels as high as 77 mg/l. Therefore, turbidity values below this range are not detrimental to the depuration process. However, excessively high turbidities may have undesirable effects.

Oysters are filter feeders and almost all of the solids suspended in the water flowing into a depuration tank are filtered from suspension by the oysters and deposited on the bottom as feces or pseudofeces. These biodeposits are a reservoir of bacteria which may recontaminate the shell stock if resuspended. These deposits constitute a potential hazard, since the oxygen demand is great. Should a malfunction stop the flow of water to a tank, dissolved oxygen levels would drop quickly, and hydrogen sulfide created by the decomposition of these deposits could kill the oysters in the tank.

Excessive turbidity will also reduce the penetrating power of ultraviolet light used to sterilize water. As suggested previously, the water used for depuration might be obtained from salt water wells and if this were the case, then suspended solids would not be a problem. We recommend that a depuration plant be located where waves, currents, or other factors do not cause excessive turbidity.

Disease

Oysters growing in Chesapeake Bay and its tributaries are subject to infection with *Minchinia nelsoni* (MSX) and *Dermocystidium marinum* (Dermo). Individuals infected with these organisms depurated as well and as rapidly as those free of infection. Therefore, these diseases do not represent a problem for depuration of oysters in Chesapeake Bay provided the oysters have not become gapers.

ENGINEERING CONSIDERATIONS

In this chapter, the various items of a mechanical nature and the unit processes will be presented. These have been grouped in three categories: water supply and treatment units, oyster processing units and depuration tanks.

A. Water Supply and Treatment Units

Water pumps. Pumps will be needed to get the water from the estuary to the plant and to provide the hydraulic head for flow through the plant. Either a single large pump or several smaller pumps would be suitable. The use of several small pumps gives flexibility and thereby allows energy costs to be reduced if a reduced flow is needed. Also, it would cost less to have a spare pump for use during malfunctions and periodic maintenance. The pump(s) selected must provide the total flow required for that plant and meet the head requirements of the particular site. The pump must be able to withstand salt water, durable and easy to maintain and repair, since the entire operation is dependent on a continuous and adequate supply of water

We recommend that the sea water be pumped to a constant head tank, then flow by gravity through the irradiation unit and finally to the depuration tanks. The constant head tank should be equipped with an overflow drain capable of handling the maximum flow possible from the pumps. The head tank need not be large, but might have a volume of 10 to 50 gallons, depending on maximum flow rate. A flow meter should be located downstream of the constant head tank to monitor the flow of water to the irradiation unit.

Plumbing. Pipes are available in a broad range of materials and sizes, and many of these are suitable for use in depuration plants. Exterior intake and drain pipes should be able to withstand environmental conditions (eg. waves, freezing) and should be sloped so that they can be drained when necessary. The design also should allow for easy cleaning and flushing by breaking it down into sections coupled together with easy-to-take-apart unions.

Fouling and accumulation of deposits are likely to cause problems with the interior plumbing. Consequently, dead spaces where the water velocity is either very low or zero are to be avoided. If the water velocity through the pipes is on the order of several feet per second, fouling organisms will have difficulty attaching to the pipe walls. However, over extended periods of time some fouling will occur in all pipes and if left unattended would clog the pipes. A simple method for removing fouling organisms is to fill the pipes with freshwater and allow to stand for a day. The freshwater will kill the barnacles, etc., which can then be flushed from the system. Unless operations are to be stopped during this period, a double system of pipes is required. One system is held with freshwater in the pipes while the other is used for plant operations.

Whether a single or double system is used, it is recommended that the plumbing system be easily altered. For example, if PVC pipe is used, it is recommended that the joints be threaded rather than cemented and that extra couplings be placed on long lines. Although extra costs will be incurred for both labor and equipment, it will be possible to modify or replace portions of the system at a later date and to clean the pipes if fouling or sedimentation has occurred.

Although shut-off valves increase head losses and can be expensive, it is recommended that a sufficient number be installed to allow the flow of water to each tank to be controlled separately. Ball valves are recommended as being durable and long lasting. Although the primary plumbing system should be of rigid materials, it is suggested that the final section to each tank be flexible. If this were the case, then the flow to each tank could be measured by simply removing the hose and noting the time necessary to fill a bucket. With this set up, it would be possible to stop the flow to unused tanks or to increase the flow to refill a tank more rapidly after daily cleaning. If the tanks are to be hosed with treated seawater then provisions for this must be included in the plumbing design. A separate set of lines with an auxiliary pump to increase pressure would facilitate these operations.

A standard freshwater system will be needed for drinking water, toilets and laboratory, if included. Additionally, tanks could be washed with potable freshwater. Wastewaters from the fountains, toilets and sinks should be sent to a municipal treatment system or on-site treatment, such as a properly designed and maintained septic system, should be provided.

Irradiation. Irradiating the water with ultraviolet light is an effective method of purification which introduces no foreign substances into the water. For these reasons it is the only method we can recommend. Several reliable ultraviolet radiation units are available commercially or units may be constructed using commercially available ultraviolet light tubes (see Figures 2 and 3).

There are other methods for purifying the inflowing water, such as pasteurization and treatment with chlorine and ozone. Pasteurization requires heating and thus would be expensive. Treatment with chlorine or ozone is not recommended because residual compounds may interfere with oyster activity. Although these methods of purification are satisfactory for many applications, they are not suitable for depuration.

It is recommended that the UV unit be in an enclosed area above the depuration tanks, along with the seawater storage tank described above. There are two reasons for this. First, this location will minimize the chance that persons unfamiliar with the unit will expose themselves or others to ultraviolet radiation. Second, by enclosing the area, the high humidity, salt air will be kept from the unit, reducing corrosion and the likelihood of shocks or short circuiting. Wiring must still be examined and maintained periodically, since some corrosion will occur anyway.

Photos of a four tube unit that was constructed at VIMS are shown in Figure 2. Figure 3 includes photos of both a commercially available unit and the so called "Kelly-Purdy" unit, a 12-tube system. These units may be constructed from readily available materials such as plywood, epoxy coatings, etc.

All essential details of UV units use and maintenance were given in Depuration Plant Design (Furfari, 1966), and therefore, the following section is quoted from that source:

> "Ultraviolet light. There are four operational items pertinent to the use of this or any other UV treatment unit. They are:
>
> 1) bulb monitoring and replacement
> 2) maintenance of the unit
> 3) safety of personnel
> 4) reliability
>
> UV tubes should be checked for intensity (commercial meters available) on a monthly basis and should be replaced when they reach a point of 60% efficiency or

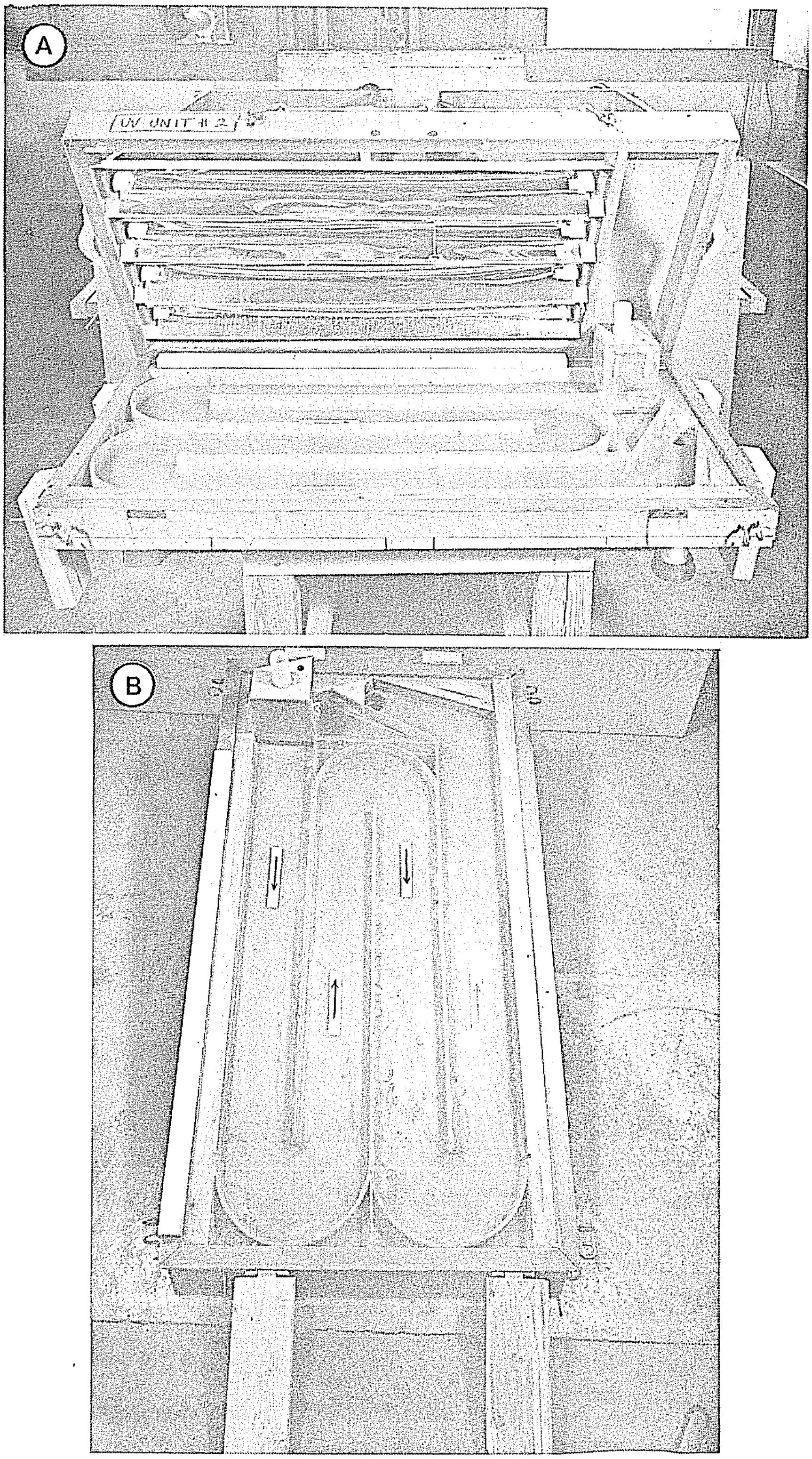


Figure 2a & b. Four-lamp ultraviolet treatment unit used in shallow tray experiments. (Described in Volume 1.)

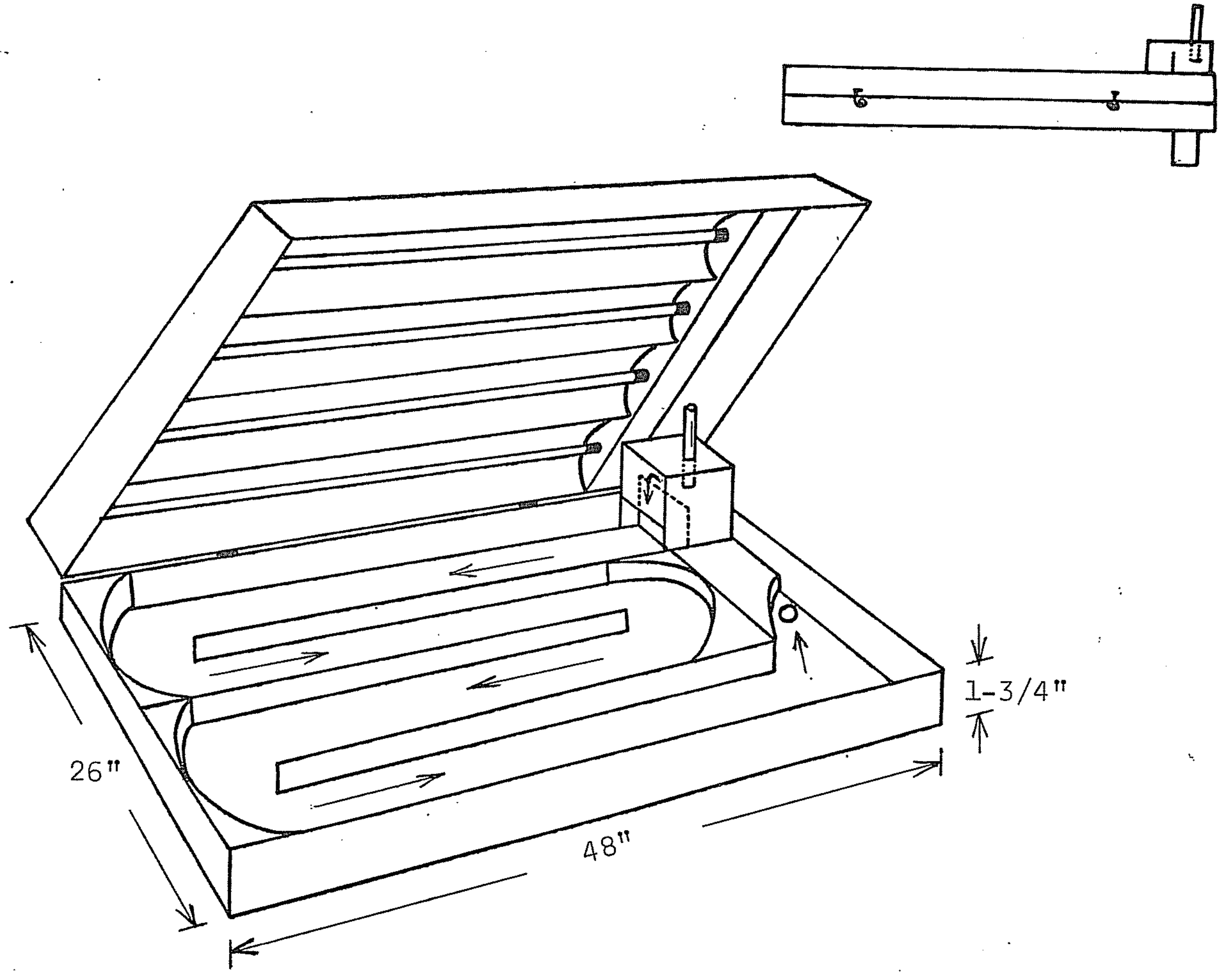


Figure 2c. Diagram of four-lamp ultraviolet unit showing construction details.

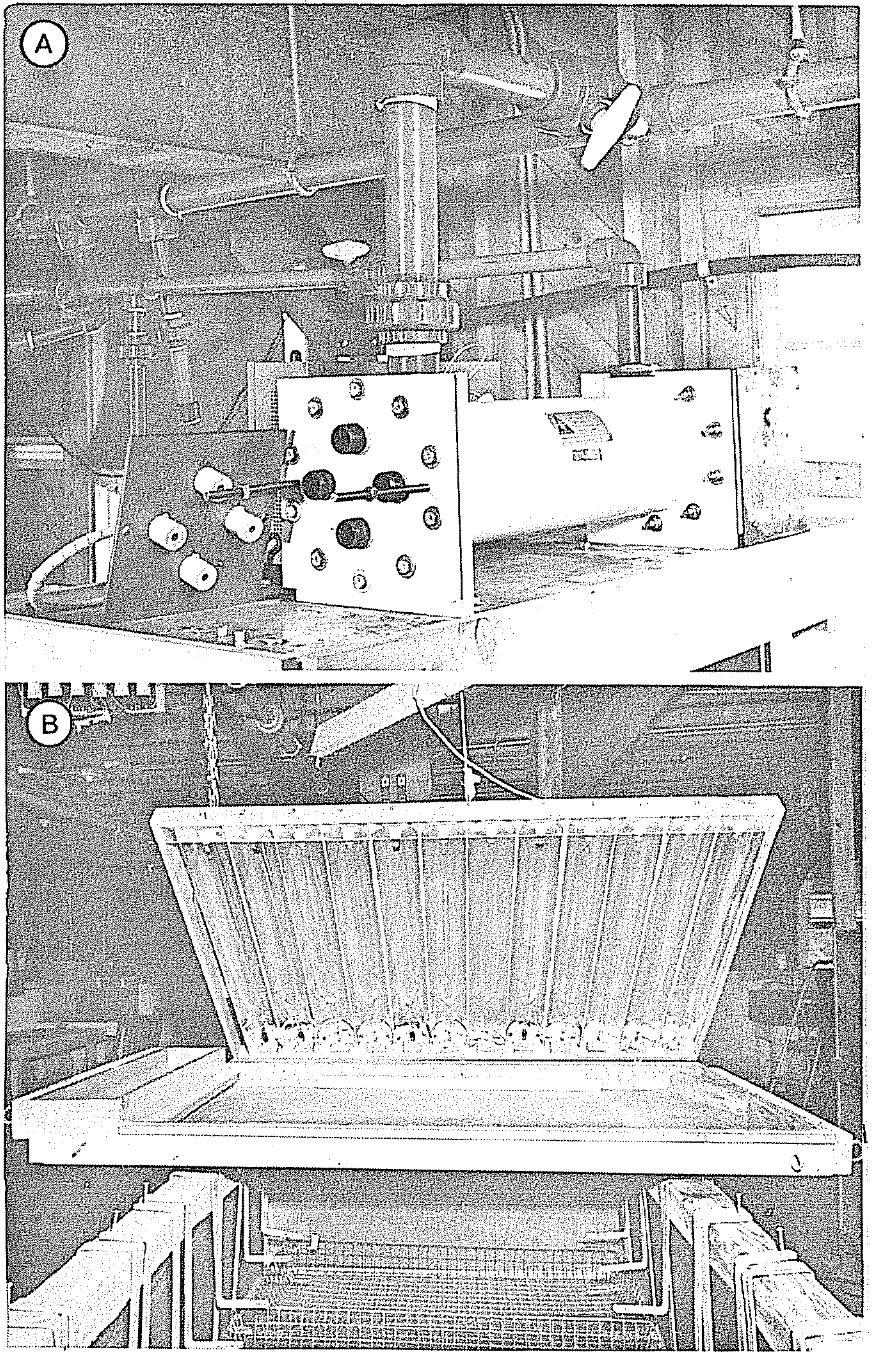


Figure 3. Ultraviolet lamp units used in experiments with commercial-size tanks. (A) 4-lamp commercial-type unit used with 2 x 8 and 2 x 4 tanks. (B) 12-lamp Kelly-Purdy type unit used with 4 x 8 tank and flumes.

> 7500 hours old; whichever occurs first. A log of intensity must be kept and an orderly numbering procedure for units and bulbs should be established.
>
> Tubes and reflectors should be cleaned daily. Cleaning can be done with a clean damp cloth and dried with a dry cloth.
>
> Signs stating, "Ultraviolet Light - Danger to Eyes - Do Not Look At Bulbs Without Eye Protection", must be displayed in full view to personnel and casual visitors. Conjunctivitis is caused by looking at lit UV bulbs, and although it is not usually permanent, it is extremely painful (Phillips and Hanel, 1960). Skin protection, especially to the face and hands should be procured for personnel monitoring the bulbs. Eye protection consists of ordinary glasses with solid side pieces. Face protection is afforded by many clear plastics but not all. Plexiglas (lucite) transmits about 5% of 2537 Angstrom light. Cellulose acetate butyrate (0.41 mm) will transmit 2537 Å to some degree. Skin protection for the head is afforded by a hat, and on the hands by thick gloves.
>
> An automatic shut-off switch is a required safety feature to the Purdy Unit (for any unit) to break the electric circuit when the lid of the unit is raised.
>
> The continuity of operation of any UV unit during a depuration run must be monitored. Since power failures occurring in the absence of personnel may not be noticed, the shellfish may not receive acceptable water during some of the process time. Inexpensive electric current on-off records are available to serve not only as a measure of bulb life. Electric clocks installed in line with the different UV units, synchronized with the main power supply offer another means of determining how long UV units were not in operation. UV photo cells and intensity meters that may activate warning devices or electric controls are useful. However, these must be used with caution since the Purdy Unit has 13 tubes separated over a distance of about six feet and monitoring all or some of these by means of photo cells may not be practical."

<u>Removal of suspended matter</u>. Removal of suspended matter from the effluent water might be required by state water pollution control agencies and/or U. S. Environmental Protection Agency. Since very few depuration plants are in operation, guidelines for this industry have not been issued. In general, if influent water is suitable for depuration purposes, it is unlikely that the effluent water will contain any contaminants other than

bacteria and solids. The solids, and therefore, probably most of the bacteria, will be present in the tank washings rather than the effluent during depuration. This water could be diverted to the municipal sewer system. Otherwise plant operators might be required to disinfect the water and remove suspended sediments before discharge to the estuary. The National Pollutant Discharge Elimination System (NPDES) permit, issued by the appropriate state or federal agency, will set the standards for the return flow.

Removal of harmful substances. In general, depuration plants should not be located where concentrations of harmful natural compounds (eg. ammonia) or by-products of man's activities (eg. radioactive particles, heavy metals, hydrocarbons, pesticides, herbicides) exceed state or federal standards or where there is a danger that oysters may accumulate quantities (during depuration) to unacceptable levels. Treatment methodologies to remove such compounds are very expensive, difficult to control and probably introduce additional compounds which would interfere with depuration. Thus, other treatment processes are not considered likely for depuration plants. For the same reasons, completely closed systems are not practical and semi-closed systems should be designed to preclude the build-up of waste products and other compounds. At a minimum, all recycled water should pass through the ultraviolet units before re-entering the depuration tanks.

Aeration. Dissolved oxygen levels in depuration tanks should not fall below 2 mg/l. In general, supplemental aeration should not be needed in a flow-through system. However, if the water supply system were to fail, respiration by the oysters would deplete the oxygen supply and decomposition of biodeposits in an anaerobic environment would produce hydrogen sulfide which kills oysters. Since oysters can survive out of water for several days, draining the tanks is a simple and easy means to protect the oysters when there is a malfunction or failure in the water supply system. Alternately, it is a relatively simple matter to pipe compressed air throughout the plant and provide aerators in each tank. If properly located, the aerators would not only maintain DO levels but would induce a circulation which would bring the oxygen to all parts of the tank. The air-induced flow should be gentle since more turbulent conditions will cause the biodeposits to be resuspended.

B. Oyster Processing Units

Storage. Like all perishable products, shellfish received at a depuration plant should be placed in a controlled storage area. A most important aspect is to avoid excessive temperature changes and if oysters are to be processed within 24 hours, they should be held at about the same temperature as that where they grow, but not higher than about 70°F (21°C).

If holding for longer periods is indicated, they should be held at temperatures and under conditions specified by State or Federal regulations. After storage at reduced temperatures, additional time might be required for depuration, since the oysters will not resume normal pumping until after they are acclimated to the higher water temperature.

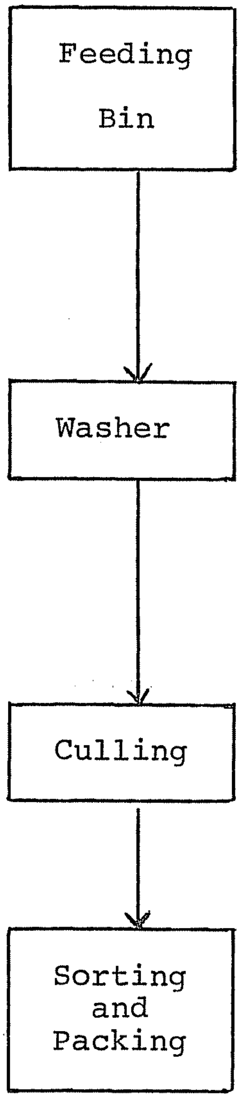
Shellfish which have been depurated should be held in cold storage prior to sale in the shell or to processing. If a single storage unit is used, it must be divided by barriers to insure that treated and untreated shellfish are not mixed.

Washing and Culling. Incoming oysters should be washed to remove mud and slime, which could provide a habitat for bacteria to multiply in the depuration tank. At the same time, the oysters can be both culled and sized. Culling is needed to remove oysters with broken shells and sick or dead oysters called (gapers) because the hinge muscle is lax and the two shells are not closed tightly. Gapers must be removed not only because they are not suitable for consumption, but also because they normally do not reduce the coliform levels in their fluids and may serve as a reservoir of contamination thereby greatly hindering depuration. Also, the washed and culled oysters may be sorted according to size as they are placed in baskets.

Upon completion of the depuration process, washing and culling should be repeated since the biodeposits accumulating on the shell stock usually are not removed completely. Also, a certain number of shellfish may die during the process, and these should be removed. Sorting would not be required the second time, but could be practiced equally well on outgoing, treated oysters as on incoming, untreated oysters.

Except for very small plants, it is expected that washing will be done by machine. For small to medium size plants, a single washer would be used for both untreated and treated oysters. In larger plants, two machines

PROCESS



COMMENTS

May be filled with water to reduce breakage when oysters dumped into bin. Serves as loading area for washer.

Oysters sprayed with clean water for 10 seconds or more to remove dirt and attached organisms. Water is under pressure and must come from all directions. A device to turn oysters facilitates cleaning.

Slow moving conveyor. Gapers, dead oysters and those with broken shells removed and discarded.

Slow moving conveyor. Oysters placed in trays to go in depuration tanks. They may be sorted by size at same time.

Figure 4. Schematic of washing and culling process.

would be needed since each would be used a significant portion of the day. These units, which are likely to cost between $3,000 and $5,000 each, move the oysters on a chain link conveyer belt through a housing wherein treated water is sprayed under high pressure from both the top and the bottom. Since oysters have hard, thick shells, it is probably not necessary to have a water bath before the conveyer, although this would not interfere with the operation. After the washer, a reasonably long section of conveyer belt should be provided to allow ample time for sorting and placement of the oysters in baskets. Gapers should be removed at this stage. Gapers and other unsuitable oysters should be treated in such a fashion to preclude human consumption, and placed in containers for ultimate disposal in a local solid waste system. A diagram of a typical culling, washing and sorting sequence is given in Figure 4.

Baskets. The washed and culled oysters will be put in baskets or trays which will then be placed in the depuration tanks. These baskets should be sturdy, durable and inert. Wood baskets are not recommended since untreated wood may provide a habitat for bacteria within the depuration tank. Exposed metal elements are also undesirable because of corrosion. Plastic or plastic-coated wire are recommended. Several types of baskets are available commercially, but these tend to be quite expensive. One type of plastic basket, approximately 12 inches by 18 inches and 6 inches deep, holds about one half bushel of shellfish, and, therefore, is easy to handle and place in the tanks. A very few manufacturers do produce coated mesh that can be cut and fabricated in a suitable size at a fraction of the cost of commercial baskets. If the mesh can be acquired and the appropriate labor is available, on-site fabrication is recommended.

No single design is recommended for the baskets. The wire mesh is recommended over other materials since the mesh impedes the flow of water less than thicker elements. Mesh size should be about one inch. Oysters should not be stacked more than 6 inches deep. Oysters at the bottom of the tray might not be able to open their shells and there is unpublished evidence from studies at the Virginia Institute of Marine Science that oysters will not depurate unless they can open up.

The baskets must be sturdy and may require a simple frame, around which the plastic coated mesh is formed. Although it is recommended that each tray be supported independently, some existing plants stack several layers of trays with 2" x 2" runners used to separate one layer from the next. Baskets and depuration tanks should be designed to fit together. The trays should fit into the tank with a minimum of space between the tray and the tank walls.

The weight of the filled basket is a very important consideration. In general, the trays will be moved manually at one or more points in the process. Thus, the combined weight of the tray and oysters should not be greater than the amount an average person can lift repeatedly. Alternately, if a completely mechanized plant is planned, the transport mechanism and the trays should be designed jointly.

C. Depuration Tanks

Four different depuration tanks, each suitable for use in a commercial operation, were tested during our studies. Successful depuration was achieved in all tanks, although some were more consistent and gave better results than others. It is our opinion that a multitude of suitable designs exists. For that reason, we do not recommend any one design above all others. However, two successful designs are shown to illustrate tank features (Figures 5 and 6). An analysis of the test data indicates that two aspects of tank design are critical: 1) residence time and 2) circulation pattern. These design points will be discussed in detail.

Residence time. The time that a parcel of water stays or resides in a tank is called the residence time. Although this appears to be a very straight-forward concept, it is difficult to quantify. For example, if a bottle of ink is poured into a tank with water flowing through, the ink will be dispersed and mixed throughout the water volume. Some of the ink will leave the tank after a very short time and some could remain in the tank for a very long time. In short, the residence time for each drop of ink will be different. The problem is a statistical one and the statistics will vary with the flow patterns within the tank. For tank design purposes, we will consider a "theoretical" residence time and compare that with dye study results.

The theoretical residence time is equal to the total water volume of the tank divided by the flow rate through the tank. Several measures of actual residence time are available, but the one used for this study is the time required for the concentration of a tracer (eg. Rhodamine WT dye) to be

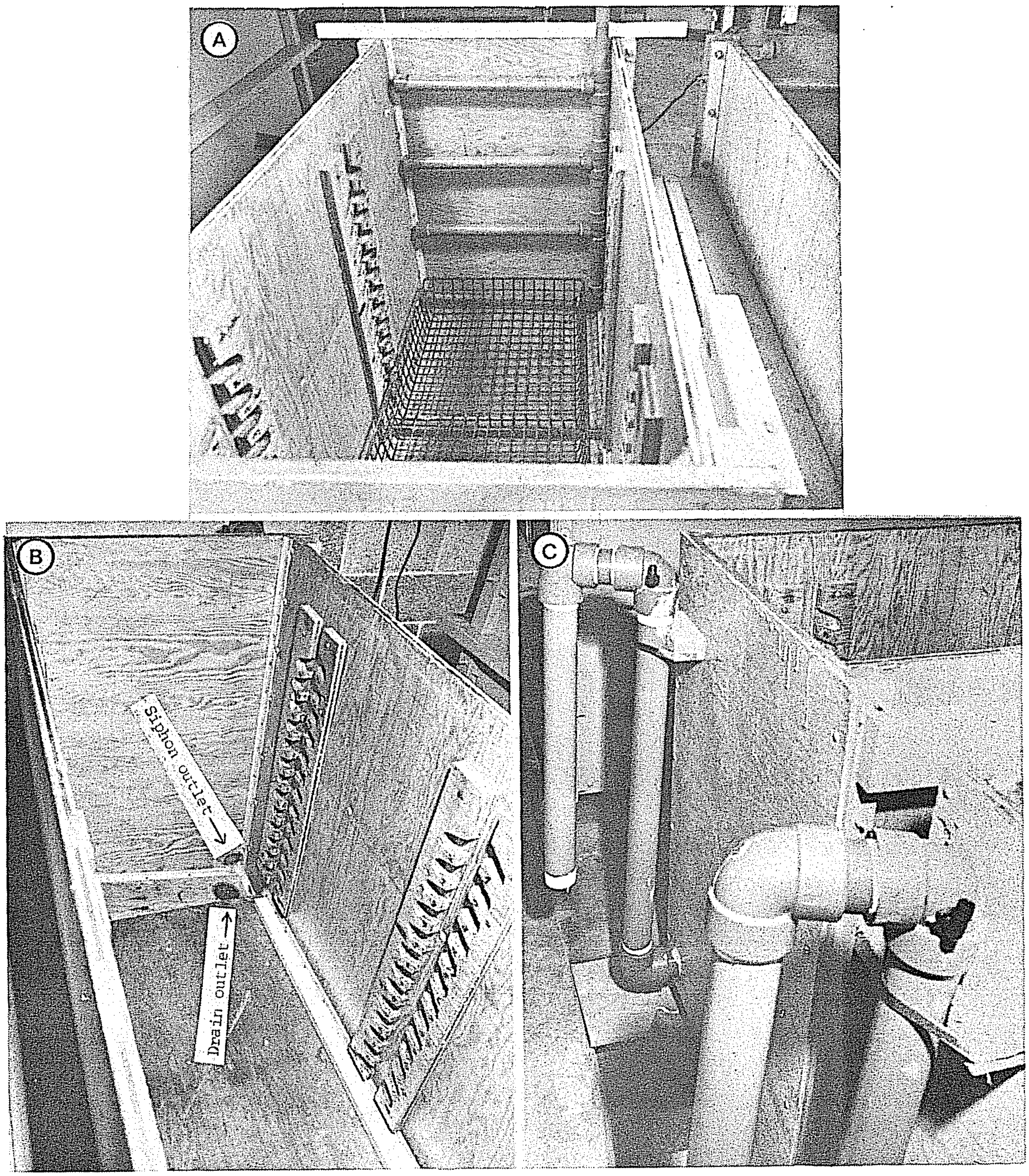
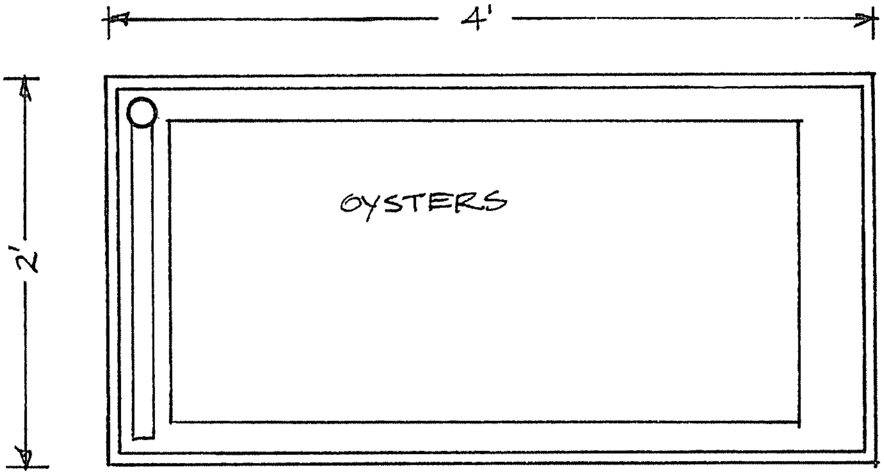
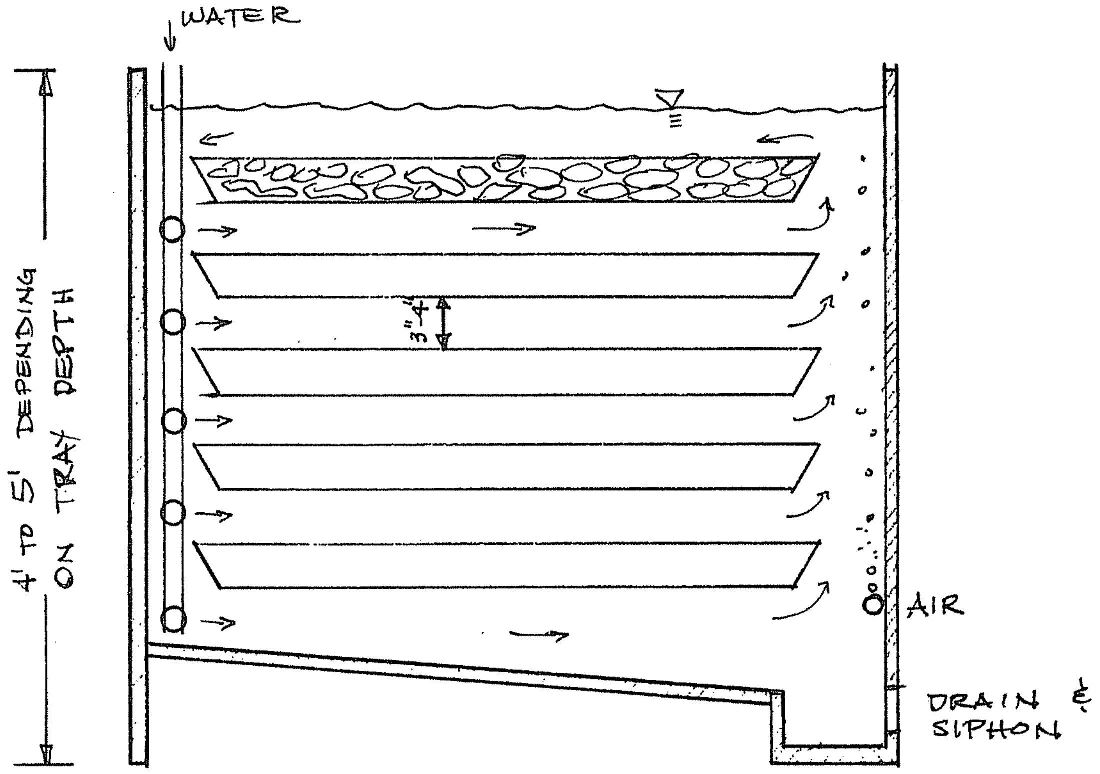


Figure 5. 2 X 4 tank. (A) Arrangement of tray and supports and inflowing water pipes. (B) Drain end showing water outlet holes. (C) Outside view of drain end showing siphon pipes.



PLAN



SECTION SCALE: 1" = 1'

CAPACITY = 3 BUSHELS

Figure 5d. Plan and section of "2 x 4" Tank.

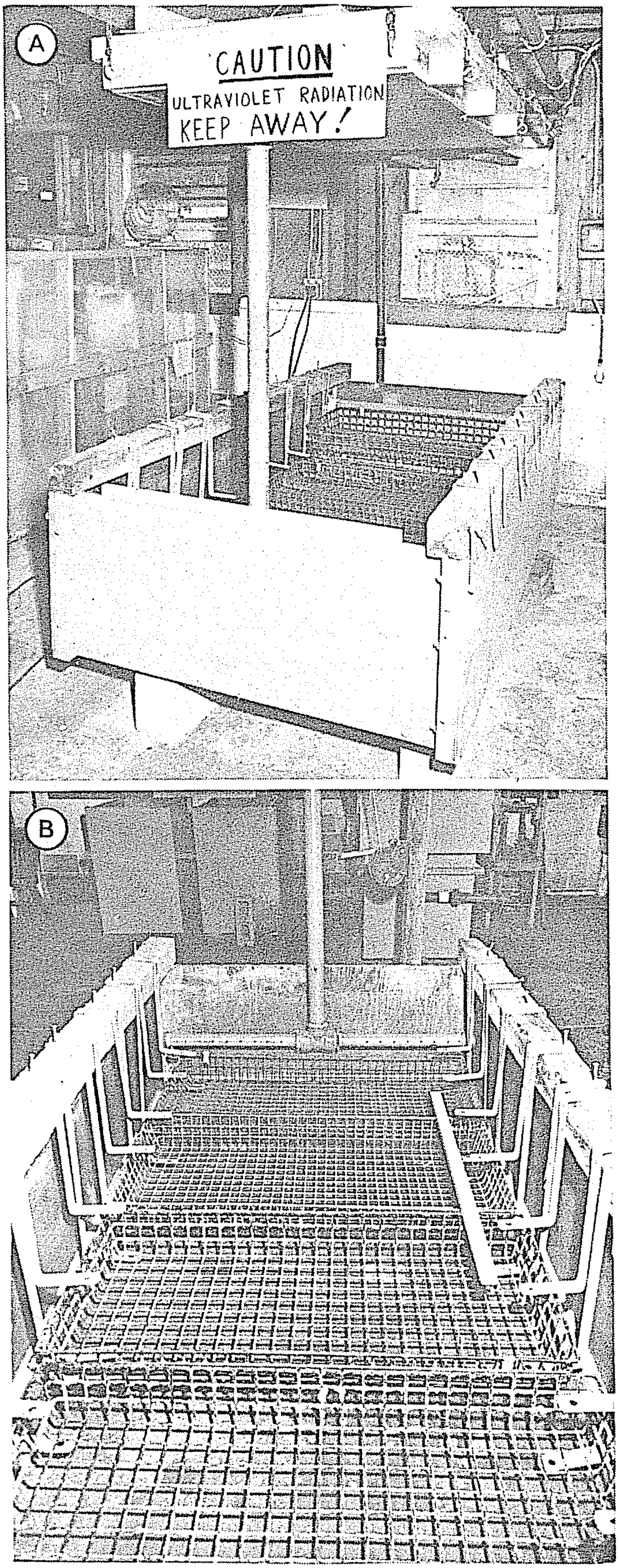
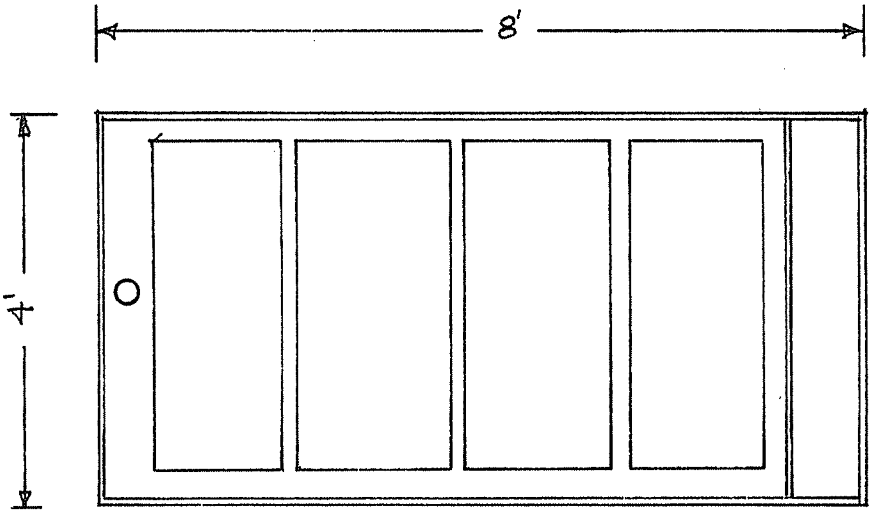
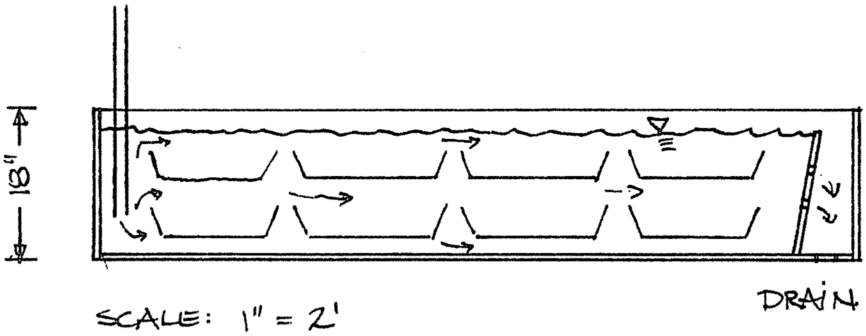


Figure 6. 4 X 8 tank. (A) Arrangement of ultraviolet lamp unit and tank. (B) Arrangement of trays, tray supports and water-inflow pipe.



PLAN



SCALE: 1" = 2'

SECTION

CAPACITY = 4.8 BUSHELS

(c)

Figure 6c. Plan and section of "4 x 8" Tank.

reduced by 90%. Table 1 gives information on the four depuration tanks which were tested.

The residence time is important because it controls, in great part, the cleanliness of the water environment surrounding the oysters. Although the water flowing into the tank approaches sterility with respect to fecal coliforms there still are coliform bacteria within the tank. They are most numerous during the first few hours of depuration, when coliform counts in the oyster are high. These bacteria will be alive and suspended in the water. Analyses of the water in the tank have shown that coliforms were present in the tank, although at low levels (normally less than 10/100 ml). This might appear to be unimportant, unless one considers the pumping rate of the oysters. It has been estimated that an adult oyster has the capacity to pump around 70 gallons of water each day. If the ambient water contained only 1 coliform per 100 mls, then the oyster could take in over 2,500 coliforms during the day. Obviously, levels must not be just low, but extremely low for the depuration process to succeed. It is essential to rapidly remove those bacteria which have been released by the oysters.

The 2 x 4 and 4 x 8 tanks are quite similar with respect to volume of water per bushel of oysters, theoretical residence time and measured residence time (see Table 1).

Circulation. Although the residence time is extremely important, it is not sufficient to simply design a tank that has a short residence time. The flow of water through the tank and around the oysters is very important as well. For example, a second dye study was conducted in the 4 x 8 tank with a small pump providing additional circulation. The residence time for this test was roughly half that of the tank with normal circulation. Similarly, the flumes have short theoretical residence times, but depuration test results for these tanks at 72 hours were not satisfactory.

A good design should provide for a flow of water to all parts of the tank and the flows should be reasonably equal. Dead spots are not good, because dissolved oxygen levels could be low in these areas and the supply of clean water to the oysters is low.

Figures 5 and 6 show the 2 x 4 and 4 x 8 tanks. Both tanks have roughly 7 cubic feet of water volume for each bushel of oysters. Although tests were not made to determine the smallest amount of water volume which

TABLE 1. DEPURATION TANK CHARACTERISTICS

	4x8	2x4	2x8	Flume
Volume - gal.	253	155	457	97
- ft^3	33.7	20.7	61	13
Vol. of oysters (bu)				
Max.	4.8	3.0	4.8	2.6
Min.		1.8	3.2	1.5
Theoretical Res.[1] Time (min)	26	26	48	19
Actual Residence[2] Time (min)	56 (30)[3]	50	60	-

[1] Max loading of oysters and unit flow of 2 gal/min/bu.

[2] Time for 90% reduction in dye concentration, interpolated to flow of 2 gal/min/bushel.

[3] With pump added to increase circulation.

would allow successful depuration, we do not recommend that this value be much lower, because there is a strong chance that the circulation within the tank will be poor. The flow rate of treated water should be no less than one gallon per minute per bushel of oysters. Higher rates will reduce the residence time and enhance the depuration process. The lower limit was determined at VIMS on the basis of studies over a wide variety of environmental parameters.

The flow of water around the oysters and between the trays should be relatively the same for all locations in the tank. The addition of air diffusers will help to maintain high DO levels and will increase the circulation. In particular, these can help to reduce "shortcircuiting" and the chance of dead spots. Air flows and locations should be selected to produce gentle flows that do not resuspend biodeposits.

Time of Depuration. Our studies indicate that oysters can be depurated in 48 hours, under the environmental conditions outlined in the preceding pages. This same time period has been suggested after research in other geographical areas. Occasionally unforeseen circumstances will cause 72 hours to be required. Bacteriological tests would determine if such a time extension is required.

If oysters do not depurate in 48 hours, the plant operator should reconsider the mechanics of his plant operation and origin of oysters he purchases. Extension of depuration time to 72 hours may yield acceptable results, but should be viewed as an indication that conditions as outlined in this report were not met. Bacteriological testing by means of Medium A-1 (see Volume I of this report) requires 24 hours to yield results; therefore, a decision will have to be made by the appropriate regulatory agency as to whether a batch of oysters can be released to the consumer before bacteriological results are known. Our research indicates that up to 93% "successful depuration" (i.e. below 50 fecal coliforms/100 g) can be expected; however, the reader must be reminded that any biological systems will exhibit variability and it would be naive to assume that 93% of the oysters will always depurate. This, coupled with human error in plant operation, will yield further variability in end product results.

SUGGESTED PLANT LAYOUTS

It is not possible to develop a single site or floor plan which will suit the needs of each and every depuration plant. In this chapter we will present a typical site plan, a schematic drawing of the hydraulic flow through a plant, and suggested layouts for two sizes of plants. These plans are for illustrative purposes and it is not intended that they should be followed exactly.

Site Plan

Transport of untreated oysters to the depuration plant may be by water or land. Transport to market, on the other hand, is expected to be almost entirely by land transportation. For these reasons, we would expect a typical site plan to look something like that shown in Figure 7. Unloading facilities and a pump house would be located at the outer end of the pier. Oysters could be transferred to the depuration plant by conveyor belt, overhead trolley or wheeled on a cart. If the latter method is used, then it will be necessary to use only ramps, and no steps, between the pier and the plant interior. Since trucks will be needed to transport the oysters to market, it is a simple matter to provide for the delivery of untreated oysters by truck as well. The plant need not be located immediately on the water or on a street, as long as the right-of-way easements allow for vehicle traffic to the plant, passage of the seawater pipes and some means of transporting the shellfish from the waterside to the plant.

Hydraulic Design

The simplest hydraulic design is to pump the seawater to a high point, and allow it to flow through the plant by gravity (see Figure 8). In this manner, only one pump is needed. We recommend that the constant head tank (with overflow drain) and the ultraviolet treatment units be located on the second floor or in a loft over the depuration tanks. The flow of water should be metered and valves should be provided to control the flow of water to each tank. The overflow from the depuration tanks, plus that from the constant head reservoir, will go to floor drains and eventually back to the estuary. It is recommended that these be simple troughs with removable gratings, to allow easy access for cleaning. The floor should be

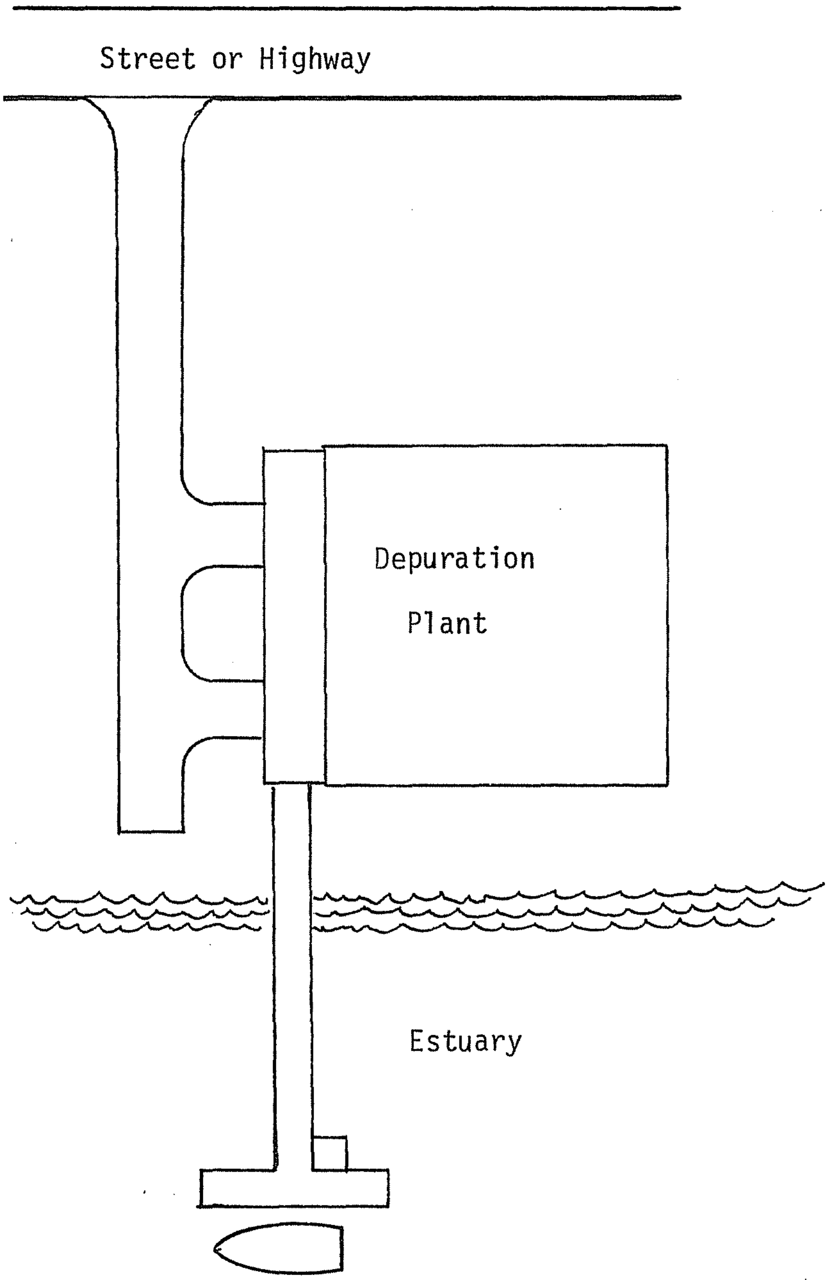


Figure 7. Site Plan.

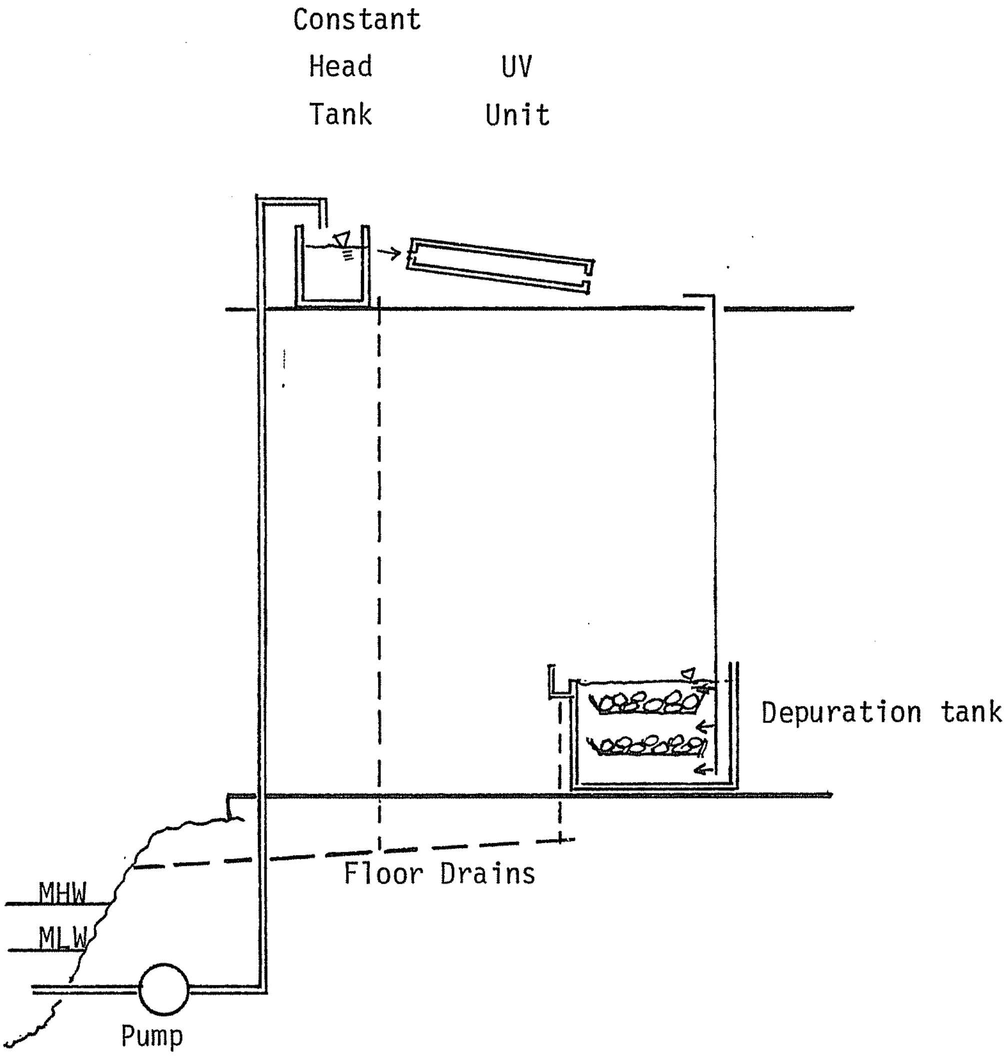


Figure 8. Hydraulic Flow Design.

sloped towards these drains to eliminate standing water and to facilitate cleaning. If the floor is concrete, we recommend that a sealer be applied which also will make cleaning easier.

Layout for a 20 bu/day Plant

Our studies did not include economic aspects of depuration, so we cannot make definitive statements regarding plant size and profitability.

Viability of a depuration plant also is a function of whether the plant is run independently or is part of a larger operation. For example, an individual might find it profitable to operate a small depuration plant (say only 10 bushels per day) using a labor pool already available to him. A completely independent operation probably needs to be larger to be profitable.

We believe that a 20 bushel per day plant would be sufficiently large to be profitable, but small enough that a single person could manage the operations with a small number of assistants. The actual daily output cannot be estimated precisely. The total capacity of our "typical small plant" is 48 bushels, or a flow of 16 to 24 bushels per day. Consequently, the water pumps must be able to supply at least 50 gallons per minute.

For this size plant a single controlled storage area and a single washing and culling machine would be needed (see Figure 9). Since the trays of oysters are likely to be transported manually, we recommend that narrow tanks and small trays be used, so that a tray of oysters could be lifted into or out of the depuration tank easily by a normal adult. The plant could be expanded either by repeating the floor plan (see Figure 10a) or extending the depuration tank area to the rear (see Figure 10b). The former would double capacity, while the latter approach could be varied to suit needs.

Constant head tanks and ultraviolet units would be located in a loft over the depuration tanks, with access by retractable steps.

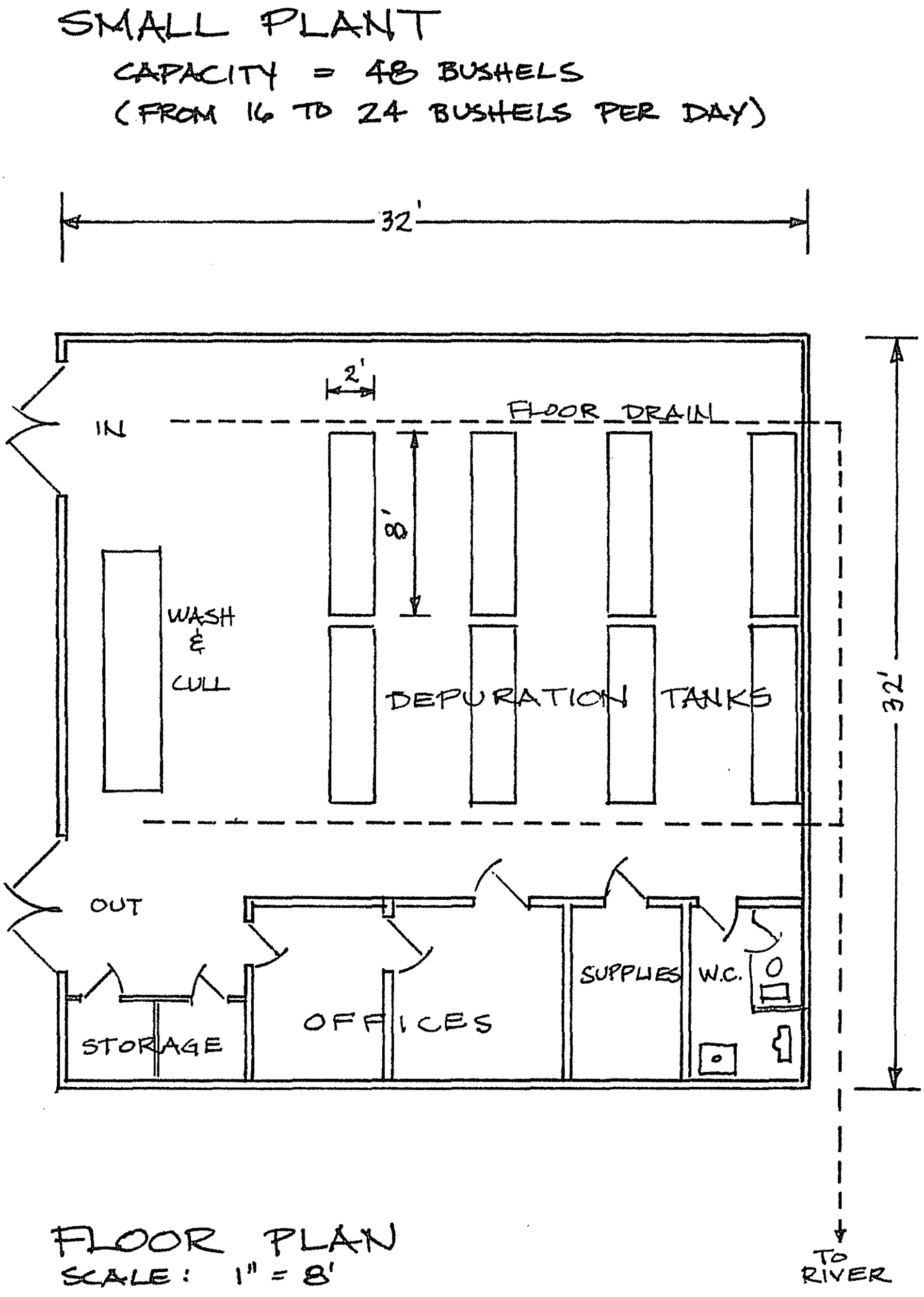
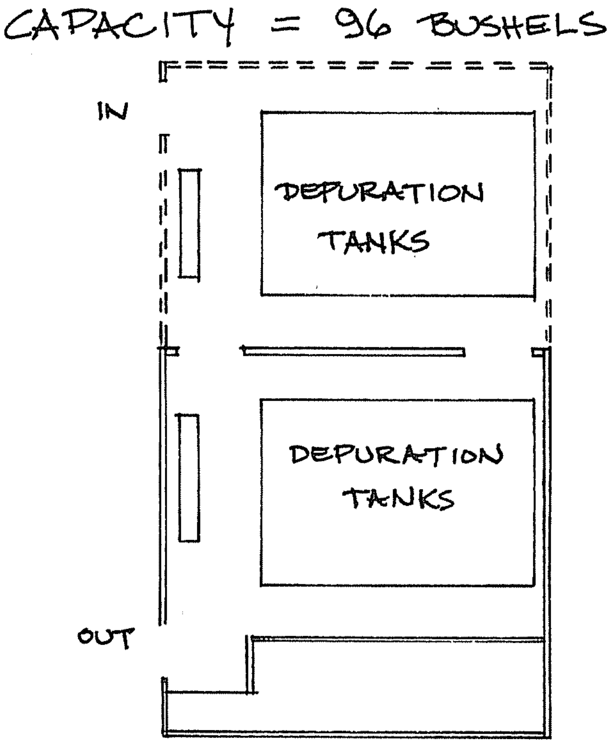
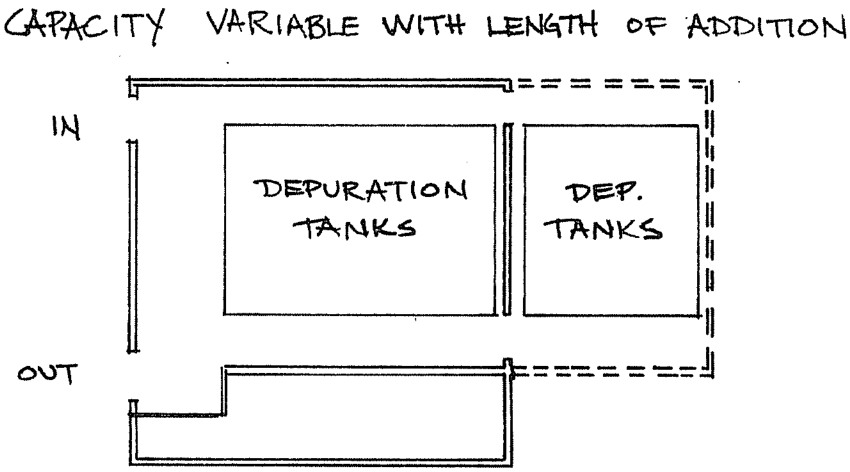


Figure 9. Layout for a small plant.



a) Layout with doubled capacity.



b) Layout with variable capacity.

Figure 10. Two modes of expansion for a small plant.

Layout for a 100 bu/day Plant

Since no oyster depuration plants currently operate in Maryland or Virginia, it is difficult to assess the potential for success of such an operation. We believe that it would be possible to operate a large depuration plant, perhaps in conjunction with a shucking or processing plant, which would handle roughly 100 bushels per day. The average capacity for the plants shown is 225 bushels, or 75 to 115 bushels per day. Plants of this size would have separate controlled storage areas and washing and culling machines for treated and untreated oysters. A laboratory for bacteriological analysis can be included in the plant if it is necessary to do so.

Plan #1, Figure 11, is a linear layout. The advantage of this scheme is that incoming and outgoing shipments are separated by around 75 feet, giving a simple traffic flow. Plan #2 is more similar to the small plant design layout and could be enlarged in smaller increments than plan #1 (see Figure 12). In each case, water pumps must be capable of supplying more than 250 gallons per minute. At least five Kelly-Purdy UV units would be necessary. The number of commercial units necessary would depend on the manufacturer's maximum rate flow, with appropriate reductions for turbidity levels which are likely to be encountered at the particular plant location.

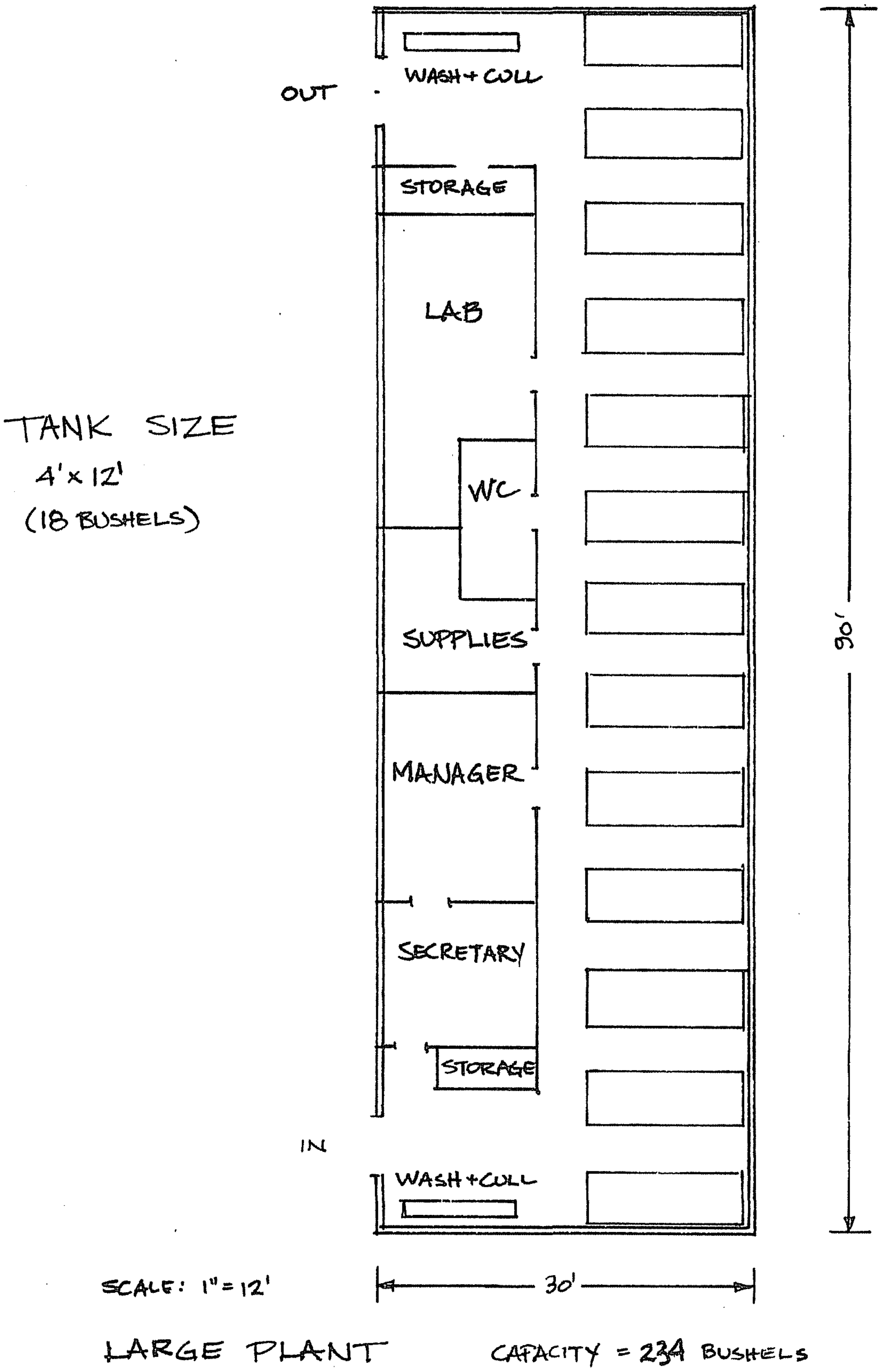
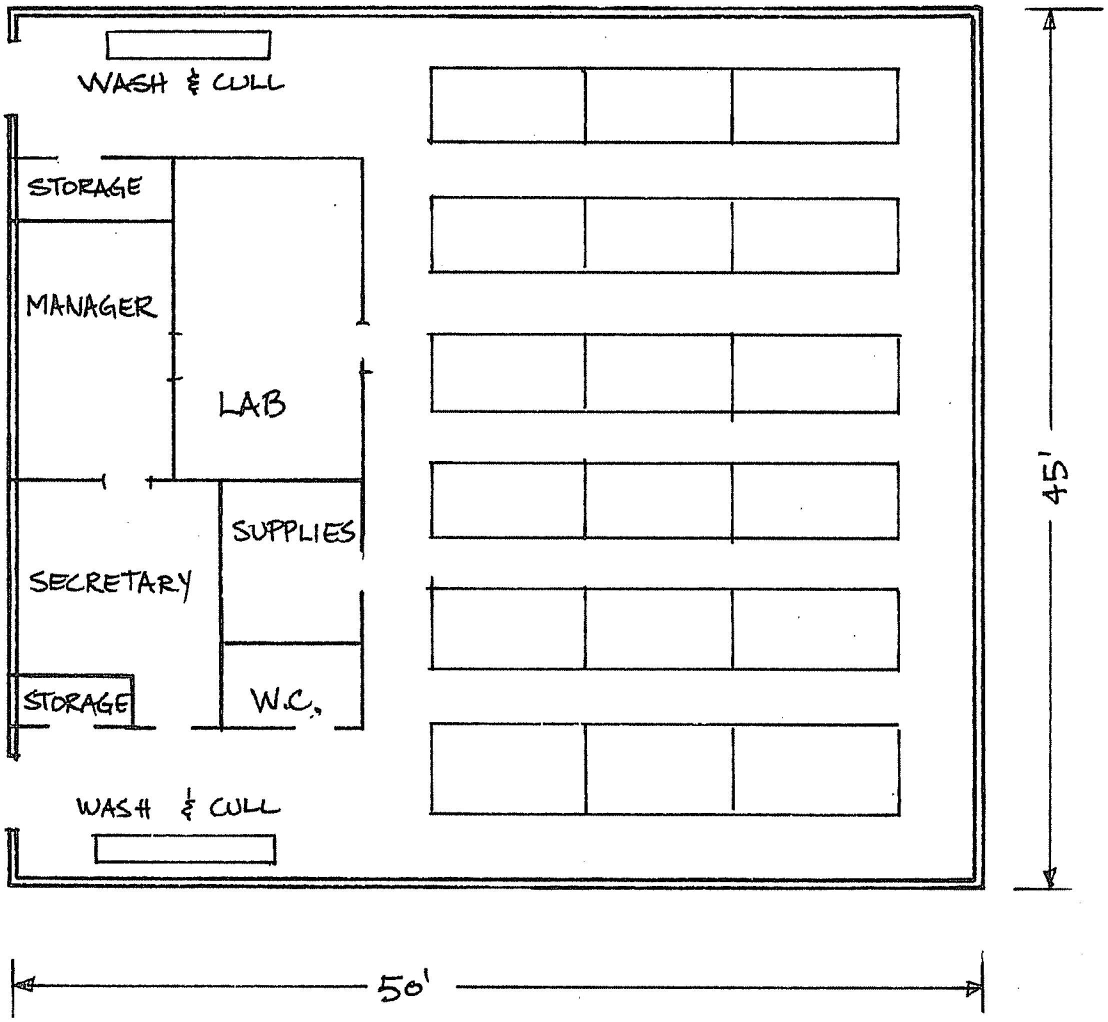


Figure 11. Layout for a large plant, linear design.

TANK SIZE - 4'x8' (12 BUSHELS)



LARGE PLANT
SCALE: 1" = 10'

CAPACITY = 216 BUSHELS

Figure 12. Layout for a large plant, square design.

SUGGESTED OPERATING AND MONITORING PROCEDURES

This chapter is devoted to procedures to monitor the depuration process. During our studies it was not possible to investigate every aspect of oyster depuration. Therefore, some of the recommendations are based on information from other studies and the authors' familiarity with oyster behavior and the waters of Chesapeake Bay. It is possible that experience will show some of the recommended procedures will be too restrictive while others will be too lax. This occurs because it is difficult to extrapolate laboratory data to a routine work situation. We have attempted to point out areas of concern which could affect the successful operation of the plant. Some of these must await detailed study at the specific sites. It is implicit in the development of a new industry that the procedures evolve with time. Thus, we suggest that monitoring during start-up of the plant be considerable, but that as the amount of information grows, the frequency and sophistication of the sampling effort be reduced.

We recommend that good records be kept for several reasons. First, it contributes to the professional nature of the work and protects the plant operator from unjust criticisms. Second, data on temperature, salinity, turbidity and dissolved oxygen should be obtained since the plant operator is not likely to know in advance typical values of those variables for each season of the year. It is for that reason that we recommend that a comprehensive data set be collected, at least during the first year of operation. Simple plots of daily or weekly readings will highlight those periods when environmental conditions are critical and special care is needed in operating the plant. During subsequent years, the frequency of sampling may well be reduced, except in instances of extreme variation. In those instances, sampling will be necessary in order to assure quality control.

In the construction and operation of the depuration plant, it is necessary that plant layout, location, construction materials, and operational procedures be in accordance with standards and codes. These include federal guidelines, such as those issued by the National Shellfish Sanitation Program, state regulations, such as building codes, and local ordinances, such as zoning regulations.

In respect to state regulations, five states now have operational depuration plants, but others have no regulations specifically formulated to regulate such establishments. Therefore, prior to establishing a plant the applicable health regulations should be ascertained from the state health department and/or food regulatory agency. In the case of interstate commerce, the U. S. Food and Drug Administration also should be consulted.

Site Evaluation

The selection of a site will be dictated by a variety of factors, including the cost and availability of land, zoning restrictions, proximity to shellfish growing areas, available labor supply and access to the site by water and land. Salinity and water quality are important for biological reasons. It has been recommended that the lower limit for salinity be 10 parts per thousand. Normally, salinities at any given location are lowest during the spring when freshwater river flow is greatest. Average values for salinity in the spring throughout the Chesapeake Bay system are shown in Figure 13.

Water quality is important since contaminants in the river water could be concentrated by the oysters during bacterial depuration and/or these materials could modify the oysters' behavior and inhibit depuration. In general, it is recommended that depuration plants not be located near industries, especially those which discharge wastewaters to the river. Heavy metals, hydrocarbons and other foreign compounds are not acceptable. Similarly, plants should not be located near large sewage treatment plants. Although ultraviolet light effectively kills coliform bacteria, it is not certain that all of the more resistant forms of pathogens will be eliminated as well. Finally, it must be noted that rural sites are not necessarily satisfactory either. Pesticides and herbicides used in modern agricultural practice may enter the waterways, and concentrations could be high during rainy periods.

In short, a comprehensive evaluation of water quality is required. Much of the information can be obtained from state regulatory agencies that monitor water quality conditions, educational institutions that sponsor environmental research and local governmental bodies. Alternately, an environmentally-oriented consulting firm could be hired to do the evaluation

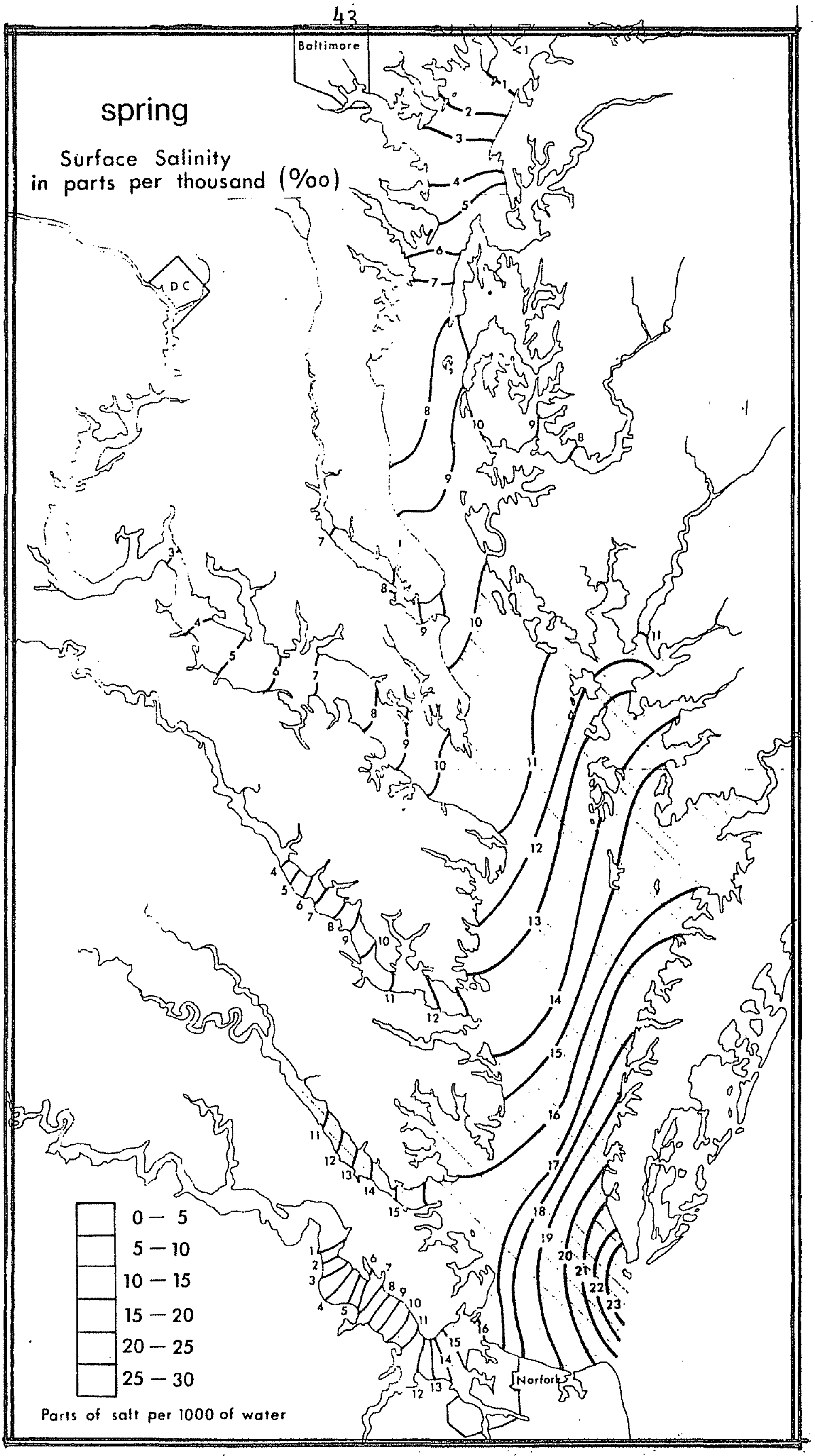


Figure 13. Spring surface salinities in Chesapeake Bay.
(From Pritchard, 1952)

and assist with the application for the NPDES (wastewater) discharge permit and other permits. In particular, permits are required for dredging and other engineering activities such as bulkheading or construction of a pier.

Water Quality Within the Plant

The quality of estuarine water flowing through the depuration system should be monitored. Fecal coliforms, temperature, salinity, dissolved oxygen and turbidity should be measured to insure that coliforms present in the incoming water are eliminated following irradiation and that physical-chemical conditions are adequate for successful depuration.

Bacteria. In constructing a depuration plant, it should be established that the ultraviolet light system is capable of reducing fecal coliform levels in water entering the depuration tanks to less than 1.8/100 ml (i.e. indeterminate levels). The precise number of samples needed to establish this fact is not known; however, it should be reasonable to obtain daily samples during the first week of operation prior to initiating depuration, and if bacterial levels are acceptable, the operator can be encouraged that his UV units appear adequate. Thereafter, the frequency of sampling is subject to conjecture. If the bulbs are replaced when burned out, kept clean and the water flow rates through the units do not change, further monitoring would not be necessary. However, malfunctions or unforeseen changes may occur; therefore, at least a weekly sample of incoming water and that leaving the ultraviolet units should be taken and tested as a quality control measure. Of particular importance, values observed for incoming water would allow the plant operator to anticipate whether estuarine water quality was changing to higher coliform levels, thereby permitting him to make adjustments, if necessary, in his purification of incoming water.

Salinity. Salinity should be measured and recorded when critical levels are suspected. If the difference in salinity between the growing area and the depuration tank is greater than 10 parts per thousand (ppt), then the oysters should be watched to be certain that they resume pumping within a few hours of being placed in the depuration tank.

Tables of specific gravity of seawater as a function of salinity and temperature are available; therefore, salinity can be measured with a hydrometer. The specific gravity of the water, combined with a temperature reading, will allow the salinity to be estimated. If the temperature and salinity ranges are great, a set of hydrometers may be required to cover the entire density range, but the cost of a single hydrometer is less than \$10. Hand held refractometers and conductivity meters are convenient, available from many sources, and cost several hundred dollars per unit.

Temperature. Temperature should be measured and recorded when water temperatures approach the recommended lower limit ($14^{o}C = 57^{o}F$) or are elevated (above $30^{o}C = 86^{o}F$). A simple laboratory grade thermometer is sufficient for these purposes. Approximate cost is ten dollars.

Dissolved oxygen. Oxygen levels (DO) in the depuration tanks should be monitored at least twice a day during summer months when both salinity and temperature values are high. At this time, DO saturation values are reduced, phytoplankton activity can cause large daily variations, and the activity of the oysters (and therefore, their respiration) is pronounced. Consequently, the likelihood of having low DO in the tanks is great. If such a condition were to exist, not only could depuration be inhibited, but in extreme cases, a large portion of the oysters might die. A portable DO meter could be used to monitor DO levels in the incoming water and in the tanks. These instruments are relatively simple to operate and can be moved to many locations within the depuration tank, providing a simple and easy check for "dead spots". Although a unit costs several hundred dollars, its use could avoid mishaps and thereby save considerable time and money over the long run. Standard chemical analyses (Winkler Titration) also could be used effectively with limited training.

Turbidity. In general, turbidity measurements are not required unless the concentration of suspended matter is high. The depuration plant operator would have to determine, with the assistance of a commercial or governmental laboratory, whether the waters near his plant have the potential of suspended solid levels exceeding 77 milligrams per liter. Even then, it might be possible to accomplish depuration. Depuration data above that value is not available.

Oysters

The volume, condition, harvest area, time of arrival and zero hour for depuration should be recorded for all oysters entering the plant. The duration that treated oysters are held in storage and other relevant comments also should be noted in the record book.

A major aspect in the operation of a depuration plant will be the sampling and analysis of coliform levels in oysters. While such operations may be carried out by a plant operator, it would be highly desirable for the samples to be analyzed by a state laboratory in a cooperative program.

It is not possible to specify a single minimum number of samples to be analyzed for quality control purposes due to the natural variations among oyster populations. At the beginning of operations, one pooled sample of six oysters from each tank (for the tank size range tested in this study) could be analyzed at the end of 48 hours. The resultant data will indicate tank-to-tank variations, differences between growing areas and other potential sources of variability. The number of tanks sampled might be reduced after this initial period if the state and federal regulatory agencies concur.

When conditions outlined in this report were met, and for all tanks except the flume, oysters depurated below 50 fecal coliforms/100 g (represented by a 6-8 oyster pooled sample) after 72 hours in more than 93% of the samples. The upper limit of fecal coliforms permissible in oysters entering the depuration tanks was not reached. It is known that values of 39,000 and 52,000 fecal coliforms/100 g depurated below 50/100 g in 48 hours.

Monitoring Harvest Areas

Federal and/or state shellfish sanitation agencies are charged with monitoring water quality at shellfish growing areas and defining the various closure zones. Measurements of bacterial levels, and perhaps other water quality measures, have been made at the important shellfish growing areas for a considerable number of years. Thus, it should be possible to define seasonal and long term trends in water quality for each area. This information should make it possible for those agencies to note areas and times of the year when shellfish could be harvested and depurated successfully. Our studies indicate that oysters from waters with 1,300 fecal coliforms/100 ml and containing a fecal coliform level of 39,000 per 100 grams will depurate within 48 hours. Additional field studies might be required to demonstrate that other undesirable constituents (eg., viruses, heavy metals, etc.) are not present or are present in sufficiently low concentration that the oysters can be harvested.

CONCLUSIONS

Results of the present study indicate that depuration of fecal coliforms from oysters is possible in the Chesapeake Bay region when levels below 50 per 100 gm of tissues are considered to represent success. Since oysters may not depurate to that level when physical and chemical conditions induce stress, adequate monitoring of those parameters in the depuration plant, as outlined in this report, are necessary. However, the range of conditions which permit depuration are sufficiently broad to permit depuration during a large part of the year and in a large part of the Chesapeake Bay.

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