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# ENVIRONMENTAL, LEGAL AND MANAGEMENT ASPECTS

# OF PROPOSED OYSTER DEPURATION FACILITY

# Part 2 of 3

Oyster Depuration Facility: Engineering Assessments

bу

Marvin T. Bond and Dennis D. Truax Department of Civil Engineering, Mississippi State University Edwin W. Cake, Jr. and David W. Cook Gulf Coast Research Laboratory

## Prepared for

# MISSISSIPPI-ALABAMA SEA GRANT CONSORTIUM Ocean Springs, Mississippi



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## Background

The U. S. Public Health Service, with the cooperation of States and the shellfish industry, has had the responsibility of assuring that raw and frozen shellfish shipped in interstate commerce are safe for human consumption since 1925. For more than 50 years this program has functioned, with reasonable success, to protect the public's health and maintain its confidence in shellfish as a food source. However, these tasks are much more difficult today than they were during the earlier years of the program. The population of the coastal areas has increased greatly along with tremendous increases in the use of estuarine waters for recreational and other purposes. These factors, and others, have caused many areas supporting the growth of shellfish to be intermittently or permanently closed to harvesting.

This phenomenon was first illustrated on the Mississippi Gulf Coast in February, 1961, when the entire Pascagoula oyster reef, comprising some 540 acres of oyster bottom, was permanently closed to harvesting. This occurred at a time when daily production was netting approximately \$1,500 per day for the fisherman.<sup>1</sup> A further example occurred in the closure of the Biloxi Bay oyster reef system in 1967. Consisting of approximately 900 acres of highly productive reefs, this area has been reported as being able to support the entire raw oyster industry on a 12-month basis.<sup>2</sup>

With respect to the Biloxi Bay system, Panagiotou<sup>3</sup> indicated that, because of expected land development and differential tidal movements, the level of wastewater treatment prior to discharge required to achieve the desired water quality of the system may be beyond the scope of present technology. Therefore, the likelihood of this system being reopened is slight at best. In addition to the closed areas of the Mississippi Gulf coast, the watershed areas affecting St. Louis Bay and Pearl River are presently experiencing development. Although these areas have remained in a relatively pristine state, degradational problems with respect to pollutional parameters must be expected to occur.<sup>1</sup>

To illustrate the severity of the problem, oyster production by Mississippi fishermen has averaged only 33,000 barrels per year since 1970. Oyster biologists estimate there are approximately 100,000 barrels on state reefs presently closed because of domestic pollution. At the present average value of \$25.00 per barrel, those oysters represent \$2,500,000 worth of a renewable resource being lost to the state each year. A similar situation exists in Alabama, although the value of oysters in closed waters is significantly less.

Before oysters from domestically polluted waters can be utilized they must be cleansed. The U. S. Food and Drug Administration recognizes two cleansing methods. One method, relaying, involves harvesting oysters from closed waters and transplanting them into approved waters for at least 14 days. This method involves a duplication of effort since the oysters must be harvested twice. The second method, depuration, involves a process of self-cleansing in an onshore facility, a depuration plant, where water which is treated by ozonation or ultraviolet irradiation flows through tanks containing oysters. The depuration process requires 48 to 72 hours with bacteriological testing to confirm that depuration has been completed.

## Justification

Each method has certain disadvantages related to economics, en-

gineering, and environmental and operating characteristics. These disadvantages must be considered before selecting either method for a particular locality. Relaying appears to be the most economically feasible for Mississippi waters at the present time. However, if population in and pollution of the coastal zone continues to increase, additional oyster producing grounds and relaying areas now available may be closed. Should this occur the future of the oyster industry will rest in depuration.

Although depuration should not be considered as a substitute for adequate pollution control, it is a technically feasible process that would allow harvesting of shellfish from most of the coastal waters, thereby providing for the continued utilization of a renewable food resource and the protection of the public health.<sup>4,5</sup>

Depuration, used successfully in Europe since 1916, was first introduced to the United States in 1921. Commercial clam depuration facilities are currently operating in Maine, Massachusetts, and New Jersey. However, no oyster depuration facilities are currently operating in the United States. Thus, data are not available upon which to make a firm decision concerning the establishment of an oyster depuration facility along the Mississippi-Alabama Gulf Coast.

Although certain design requirements for shellfish depuration facilities have become fairly well-defined through the years,<sup>6,7</sup> aspects of the total depuration system have not been examined as extensively.

Representatives of the Mississippi Marine Conservation Commission, the Mississippi Research and Development Center, the Mississippi State Board of Health and the Gulf Coast Research Laboratory have met on several occasions to discuss the problems of harvesting and cleansing oysters from closed areas. Further, the Gulf Coast Research Laboratory has developed a research and development program which may culminate in the establishment of a commercial oyster depuration facility. This program is divided into three steps.

Step One is a feasibility study of oyster depuration including an assessment of the following factors: A. Environmental aspects, B. Legal aspects, C. Management aspects, D. Economic aspects and E. Plant design and construction costs. The results from Step One will indicate whether the program should proceed to the next. Part <u>A</u>, <u>B</u>, and <u>C</u>, of Step One are being assessed under Sea Grant No. 04-6-158-44060 of the Mississippi-Alabama Sea Grant Consortium (1977).

# Scope of Work

The problems addressed in this report are related to the engineering aspects of oyster depuration. Since no commercial oyster depuration plants are in operation in the United States today, real data related to the construction and operation costs are not available. Further, all former projections on depuration costs have been based on flow-through depuration systems with no wastewater treatment. Because there are significant periods of time along the Mississippi Gulf coast when turbidity is excessive and salinity is very low, recirculating (closed) water (depuration) systems with waste treatment facilities may be necessary. It is essential that a best estimate of costs associated with the development of a depuration facility be available before the economic aspects are evaluated.

Adequate data are available to permit the design and operation of depuration facilities with one major exception. A review of the literature indicates that there may be significant degradation of depuration waters returned to the estuary. There are references stating that the amount of degradation may be only minimal for effluents from flow-through

depuration plants, with assumptions that the effects may be of much greater consequence for closed or recirculatory systems. 4-7

Engineering aspects covered in the scope of work for this project are:

- A. Evaluation of the extent of the degradation of water used in both open- and closed-system depuration facilities when ozone is used to control the bacterial quality of process water.
- B. Estimation of the requirements for wastewater treatment and size of various components of a wastewater treatment plant.
- C. Provision of a design for a 100 bushel depuration facility in sufficient detail to permit an accurate estimate of the costs of construction and operation of the facility. It is not intended that the proposed design include a final set of working plans and specifications for bid purposes.

The need for oyster depuration facilities is based upon the necessity to protect the health of that segment of the public which consumes raw or partially cooked shellfish. These shellfish may contain pathogenic organisms (e.g., viruses) which were removed from their polluted environment and concentrated through normal filter-feeding activities. Those organisms may be removed from polluted mollusks in onshore depuration facilities or by offshore relaying of the mollusks to approved growing waters.<sup>8,9</sup>

# Accumulation of Microorganisms

Kelly, et al.<sup>10</sup> found that in the eastern oyster, <u>Crassostrea virginica</u>, that coliform accumulation rates varied widely within apparently similar conditions. Though correlation of these variations to individual differences of the oysters or to changes in seawater characteristics was not undertaken, oyster-water coliform ratios as high as 30 to 1 were found to occur. However, the rate of accumulation of <u>Escherichia coli</u> was found to be seasonal and the highest average ratio was determined to be 16 to 1. This highest ratio occurred in the late fall immediately after a rapid decline in water temperature to a level below 20°C and continued for several weeks until water temperatures dropped below 15°C. Lower temperatures decreased pumping sometimes to the point of hibernation.

Mitchell, et al.<sup>11</sup> and Akin, et al.<sup>12</sup> confirmed those findings for coliform bacteria. They also determined that, though the degree of concentration of type I polio virus was to a degree less than that of <u>E. coli</u>, the mechanism appeared to be the same. In addition, they found that the degree of accumulation for both was reached within approximately

four hours of exposure. This has its greatest impact when we consider the effects on oysters of discharging raw or partially-treated wastewater into growing areas. The probability of problems increases in that exact natural conditions have not been obtained in the laboratory. It is thus felt that shellfish may have an even greater ability to concentrate virus present in their environment than has been indicated in the literature.<sup>13</sup>

The actual mechanism of coliform and viral uptake appears to be the same as that of food particles, beginning with the mouth and progressing through the esophagus into the stomach and finally into the digestive gland.<sup>14</sup> Although it appears that there is no viral multiplication during residence within the oyster, a protective action of the tissue against virus-inactivation tends to allow long term residence when the oyster is not purging.<sup>15,16</sup> Thus it seems more likely that, during various outbreaks of diseases that were linked to oyster consumption,<sup>17-19</sup> shellfish were polluted in the late fall when viral uptake was optimal. The temperature then dropped suddenly to a level which probably forced feeding to stop completely, causing the shellfish to retain the pathogens for an extended period of time, thereby, serving as a constant source for the dissemination of hepatitis viruses.<sup>13</sup>

# Accumulation of Other Pollutants

The marine dinoflagellate, <u>Gymnodinium breve</u>, has been strongly incriminated as the primary source of toxin(s) present in oysters exposed to "red tides." In aquaria experiments,  $^{20}$  it was determined that a high toxicity existed in oysters after nine hours of exposure to <u>G. breve</u>. It was also found that the method of accumulation and elimination was the same as that of any food source. Therefore, the toxicity of the oysters was greatly reduced in 36 hours when they were placed in a clean environment. The accumulation of trace metals by oysters to levels much higher than that of their environment has also been established. A sample of oysters from the South Atlantic and Gulf coasts contained average copper and zinc concentrations of 19 and 230 mg/kg of wet oyster tissue, respectively.<sup>21</sup> It was also determined that chromium (III) and chromium (VI) were concentrated by oysters. Additional studies<sup>15</sup> which examined the uptake rates for lead, copper, cadium, and zinc indicated that the rate and degree of heavy metal accumulation varied for shellfish specie and pollutant. It was observed that, of the various anatomical areas studied, the muscle, mantle, mantle edge, gill, gonad, and liver tissues accumulated increased amounts of lead in the order given. With regard to the elimination of heavy metals, it was found that depletion is a slow process again varying with specie and pollutant. For example, oysters having a zinc concentration of approximately 2,000 ppm showed no appreciable decrease in concentration after 14 weeks in a flowing, zinc-free, seawater system.<sup>22</sup>

# Factors of Microbial Elimination

Just as the environment of the oyster is important in the accumulation of pollutants, environmental factors are important in the elimination of those same pollutants. The literature  $^{4-7,12,23-26}$  has identified the major factors as temperature, salinity, turbidity, dissolved oxygen, flow rate pH, and the concentration of pollutants in the depuration waters. Each of those parameters in turn had a marked effect on the time required for purification of the oysters to an acceptable level.

Torpey, et al.<sup>27</sup> found that, in general, acceptable coliform levels were reached within 24 hours. In their experiment, the initial coliform concentrations in the oyster meat ranged from 3,300 to 240,000 MPN/100ml.

Mitchell<sup>11</sup> confirmed those results but further indicated that minimum concentrations of <u>E. coli</u> and polio virus were reached during 72 hours of depuration. Other experiments<sup>12, 26, 28</sup> confirmed findings for types 1 through 3 of the polio virus contingent upon proper environmental factors.

<u>Temperature</u>. Water temperature was earlier implicated as a controlling environmental factor in the cessation of accumulation of microorganisms. Similarly, Shuster<sup>15</sup> found that the retention of viruses by the oyster for a period of 60 days occurred when temperatures decreased to 5°C. It has been found that temperatures as high as 8°C will reduce purging to an almost undetectable level.<sup>26</sup>

Presnell, et al.<sup>25</sup> compared changes in coliform levels in oysters for warm (26.9-27.7°C) and cool (18.9-19.9°C) water. They found that after four hours of depuration only 32.7 percent of the coliforms were removed in cool water as opposed to 99.5 percent in warm water. After 48 hours the degree of removal was 99.9 percent versus 93.3 percent for warm and cool water, respectively, thus indicating the effect of relatively minor temperature changes. Lin, et al.<sup>26</sup> examined the removal of type 1 and 3 polio virus and coxsackie virus B-4 at the 15 and 20°C levels and found a similar lag in the rate of depuration. With those facts in hand, sanitation personnel recommend that depuration take place at a minimum water temperature of 10°C (50°F) and a maximum water temperature of 25°C (77°F).<sup>6,17, 24, 29</sup>

Presnell, et al.<sup>25</sup> examined the water temperature relationship between harvest area and depuration facility and found that oysters removed from warm waters and placed in cooler water eliminated coliforms at a much slower rate than those placed in warm water. Conversely, they found that oysters removed from cool waters and placed in warmer water exhibited a higher rate of elimination than those placed in cool water. Furthermore,

the influence of the temperature changes was most noticeable during the first 24 hours of exposure. These findings indicate the possible benefits to incorporating a water temperature control device into depuration facilities so as to insure water temperatures equal or greater than those found in harvest areas.

However, spawning may be induced by placing ripe oysters into water warmer than they were previously in or by suddenly increasing the water temperature.<sup>6</sup> Although it is not certain whether spawning interferes with the depuration process, it is apparent that it could cause operational problems. With regard to the use of ultraviolet treatment of water in a closed system, spawning would be expected to cause marked increase in turbidity which would interfere with the disinfection process. Additionally, spawning would increase the concentration of organics in the depuration waters.

<u>Salinity</u>. There is a definite correlation between salinity and the elimination of microorganisms. Furthermore, that relationship is based on the natural environment of the shellfish to be depurated. Reduction of salinity to a level 50 to 60 percent of the oyster's natural environment was found to stop the functioning of the shellfish.<sup>26</sup> For the eastern oyster, it has been found that salinities in excess of 16 ppt yielded significantly higher coliform elimination rates while salinities below 7 ppt greatly reduced elimination.<sup>25</sup> Although this study did not relate salinity variations to the natural environment of the oysters examined, it did point out the effects of prolonged, wide-range variations. On the other hand, Cummins and Presnell<sup>29</sup> indicate that short-term variations should have a minimal effect on the depuration process. The literature further indicates that variations in salinity within ±20 percent of that found in the harvest area have a minimum influence on the cleansing process.<sup>6,17,24</sup>

These facts indicate the need to either locate depuration plants such that influent water is drawn from the areas harvested or incorporate a method of salinity control into plant facilities which would be nondeleterious to the activity of the shellfish. Such a salinity control method would allow establishment and continuous operation of depuration plants in areas subject to recurring periods of low salinity, such as those experienced in areas of the Gulf coast.

Turbidity. Design criteria outlined in the literature places a limit on depuration water turbidity of 20 Jackson Turbidity Units (JTU's). In general, this limit was established as a control on the suspended solids concentration in those water systems which used ultraviolet light for disinfection. Miescier and Presnell $^{30}$  investigated this problem using seawater with marine silt added, giving an average turbidity of 69.4 JTU's, and seawater which was filtered, giving an average turbidity of 8.8 JTU's. They detected very little difference in the efficiency of removal of <u>E.</u> coli at those two levels. In fact, they found that the rate of removal was better for the more turbid water. However, because of differences in temperature and salinity in their experiment, little if any inference could be drawn from their data other than the fact that moderately-to-high turbidity levels are not a deterent to the function of the oyster. Hamblet, et al. found very similar results for even higher levels of turbidity with respect to polio virus. Finally, Furfari<sup>6</sup> reported that significant reduction of coliforms was achieved at a turbidity level of 100 to 130 JTU's.

On the other hand, turbidity can adversely effect other aspects of the depuration process. It is thought that it may render shellfish less palatable.<sup>6</sup> High turbidity, caused by high suspended solids, can cause operational problems for depuration facilities in the form of decreased pump-life and increased solids deposition in piping and tanks. Reduced

disinfection efficiency of ultraviolet light reactors, to the possible extent of requiring an alternative method, is also caused by excessive turbidity.

Several methods exist for reducing turbidity. Those techniques include settling, filtration, and centrifugation. However, because depuration depends on gut-purging, other oyster functions and on food availability, it is important that the technique employed does not remove food particles from the depuration waters.<sup>6</sup>

<u>Dissolved Oxygen</u>. The level of dissolved oxygen in depuration waters has been shown to be important because below 2.55 cc/liter (3.6 mg/liter) the oxygen consumption of <u>C. virginica</u> becomes restricted.<sup>25</sup> Furfari,<sup>6</sup> using certain established principles and assuming a flow rate, calculated a minimum dissolved oxygen level of 5 mg/liter. This latter value has apparently been adopted as a minimum standard.<sup>17,24</sup> However, this value was based on a flow rate determined for 15°C while at the same time was based on an oxygen consumption rate found at a higher temperature; therefore, this value appears to have a safety factor built into it.

There are a number of techniques which may be employed to elevate the level of dissolved oxygen. They include diffused aeration and cascade aeration. The latter involves the free-fall of water over a certain distance or over a series of steps causing entrainment of air. Diffused aeration is the distribution of small air bubbles throughout a volume of water using a compressed air system. Each has advantages and disadvantages.

<u>Flow Rate</u>. The degree of oxygen depletion is directly related to the flow rate. The greater the flow rate the more oxygen provided to the shellfish and the lower the depletion experienced. Presnell and Cummins<sup>32</sup> examined flow rates ranging from 0.5 to 5.0 liters/oyster/hour. They found that with

proper system design and favorable environmental conditions the extents of coliform elimination by the eastern oyster was comparable at all flow rates tested. However, because of variations in environmental conditions and removal efficiencies at various times during depuration, a flow rate of 1 liter/oyster/hr was recommended. That value closely correlates with the work reported by Furfari in his calculation of minimum flow rate.<sup>6</sup> Using the value of 1 liter/oyster/hr, assuming 500 oysters per bushel, and applying an appropriate safety factor to prevent complete depletion of oxygen, a value of about one gallon/min/bushel of oysters (125 l/min/m<sup>3</sup>) was determined.

pH. The literature indicates that oyster depuration will occur between pH values of 7.0 and 8.4 units.<sup>6,24</sup> Loosanoff and Tommers<sup>33</sup> examined the effects of lowering the pH with regard to <u>C. virginica</u> and found that pH levels of 6.75 to 7.0 caused a few hours of vigorous pumping followed by a reduction in pumping. Lower pH levels caused decreased pumping while higher levels allowed normal pumping. Galtsoff<sup>34</sup> compared oxygen consumption to pH and found a major reduction in the oxygen utilization for <u>C. virginica</u> at values below 6.7.

<u>Pollution Concentrations</u>. As reported earlier, shellfish have the ability to concentrate microorganisms of which pathogens are of particular concern. Additionally, heavy metals and various organic compounds can reach levels in shellfish many times higher than the level found in their growing waters. That same concentrating mechanism causes retardation of the depuration process if the pollutant to be removed is present in system waters. Finally, any detrimental components of the depuration waters not found in the harvested area may be deposited in the shellfish to a major degree.

The literature indicates that an upper limit of 1 MPN/100 ml is the maximum colliform content of depuration process water. With regard to

metallic ions and compounds, pesticides, detergents, radioisotopes and marine toxins, concentrations are loosely limited to that of "normal" seawater such that concentration of those components by the depurating shellfish does not reach a level deemed unacceptable by the regulations of the Food and Drug Administration.<sup>6,24</sup> The literature does not indicate what levels of concentration would cause such an occurance; therefore, every effort must be made to prevent those pollutants from entering the system.

### Facility Design Considerations

There are many parameters to be considered in the construction and operation of depuration facilities. Furfari<sup>6</sup> and the U.S. Public Health Service<sup>24</sup> have extensively examined many of those parameters including aspects of oyster harvesting, transportation, storage, depuration, and marketing. Many of the guidelines established in those reports are quite thorough and the reader is directed to those publications for information supplemental to this report. However, there are considerations which we wish to further examine in this report in regard to the design and construction of oyster depuration facilities.

Depuration Water Source. As indicated earlier, the pollution status of a water source is very important. There are four basic approaches which can be taken to insure that water influent to a depuration system is of highest quality. The first involves withdrawal of water from a polluted source and subsequent treatment in additional facilities so as to provide satisfactory removal of the undesirable pollutants. However, the construction and operation of such facilities could be cost prohibitive. Additionally, the consistent removal of certain pollutants may not be technically feasible at this time on the scale required for oyster depuration.

The second approach would be the location of depuration facilities such that the surface water source is not exposed to wastewater discharges and does not receive runoff from land to which pesticides have been applied. To accomplish this task, detailed analysis of past, present, and future land use and water applications would have to be performed for the watershed area in which facilities could be situated. A complete characterization of the waters for all seasons would be required before a final location could be established. However, unless legal action is taken in regard to the future uses of land and water, there would be little guarantee that the conditions of the water source would not deteriorate with time.

The third alternative involves the use of a ground water source. MacMillan and Redman<sup>35</sup> utilized this technique in their investigation and found it to be extremely effective offering several advantages when compared to the surface water source they had at their disposal. Those advantages included:

- <u>Constant salinity</u> (24.0-25.5 ppt) thereby eliminating the need for continuous monitoring and adjustment of the salinity,
- <u>Constant temperature</u> (12.5-13.1°C) on a year round basis, therefore eliminating, or at least reducing, heating requirements,
- Minimized disinfection requirement because of the almost undetectably low bacterial content of the water,
- 4. <u>Elimination of fouling organisms</u> and growth within the seawater distribution lines due to the natural filtering action of the sand, and
- 5. <u>Elimination of pretreating water for solids</u> in that virtually no suspended matter was contained in the water.

However, certain aspects of this approach must be examined on a case-by-

case basis. Factors such as underlying strata, maximum pumping rate causing minimum draw-down, and the quantity of water available must all be taken into consideration before location of the well can be achieved.

The final technique which can be applied, and possibly the most difficult to justify, is the use of artificial seawater. Allen, et al.<sup>36</sup> presented two formulations which can be used for this purpose. There are also several commercial mixtures which could be utilized. However, the cost of water and chemicals for depuration scale operations makes this approach prohibitive at best. Only in comparison with the first approach discussed could this method find application.

<u>Disinfection</u>. Several techniques exist which effectively reduce bacterial and viral populations of waters. Each of those methods has advantages and disadvantages upon which a decision must be based with regard to the selection of disinfection processes to be incorporated into depuration facilities. The three most widely used techniques are chlorination, ultraviolet irradiation, and ozonation.

Chlorination. Several investigators have attempted to utilize chlorination with some degree of success. However, Kelly, et al.<sup>10</sup> and Galtsoff<sup>37</sup> indicated that chlorinated waters inhibited shellfish activity to the point of complete cessation of activity even after dechlorination. Allen, et al.<sup>36</sup> examined the use of chlorine in disinfecting waters to be reused in a depuration system. They found that neither breakpoint chlorination nor the reduction of chlorine residual through chemical addition would be satisfactory because of reduced shellfish activity. They decided that precise chlorine addition followed by prolonged aeration would accomplish the goal of bacterial elimination without significantly effecting shellfish metabolic activity. However, they noted that the rate of reduction of chlorine residual depended on shellfish activity thereby implying a reaction with metabolites produced by the shellfish. The use of chlorine in a depuration facility could be an operational nightmare with too high of a chlorine dose causing reduced shellfish activity, which in turn would cause an even higher residual, and with too low a dose causing an insufficient reduction in coliforms.

Ultraviolet (UV) Irradiation. The effectiveness of using UV light for disinfection in oyster depuration facilities has been well documented.<sup>38-41</sup> With proper operation of the UV system, relatively high concentration of coliforms and various viruses have been reduced to almost undetectable levels. However, proper operation of the UV system is not necessarily easily obtained.

The major deterrent to UV disinfection systems is turbidity. Levels as low as 20 Jackson Turbidity Units (JTU's) have caused minor inhibition of the disinfection process with levels of 43 to 82 JTU's causing a notable reduction.  $^{38,42}$  However, it is known that waters with turbidity levels of up to 130 JTU's can be adequately treated with proper adjustment of water depth and flow rates.  $^{42,43}$  Huff, et al.  $^{40}$  found that most river waters and other sources containing high turbidity, organics, and/or iron contents could not be satisfactorily disinfected.

These facts point out that incorporation of a UV system in an oyster depuration facility may require the addition of other treatment processes to the total system. Depending on the water source used, additional facilities for removal of solids may have to be incorporated. The increase in organic metabolites from oyster activity may also have to be reduced if waters are to be recirculated through the depuration system for extended periods. On the other hand, these treatment facilities may have to be incorporated because of other factors. If this is the case the utilization of a UV system appears to be the most economical approach to the disinfec-

tion problem for depuration plants.<sup>41</sup>

Ozone. The germicidal properties of ozone have been attributed to its high oxidation potential, approximately twice that of chlorine.<sup>44</sup> The literature also indicates that by-products from the decomposition of ozone are also effective in reducing the microbial concentration of waters. The efficiency of this disinfection technique has been well established.<sup>44-46</sup>

In addition to the disinfectant nature of ozone, several other benefits have been reported which are of particular interest for depuration systems, especially if seawater is to be recirculated.<sup>44-49</sup> These include the reduction of seawater concentrations for such parameters as suspended solids, turbidity, color, chemical oxygen demand, biochemical oxygen demand, organic carbonaceous and nitrogenous materials, ammonia, nitrites, phenols, cyanide, detergents, pesticides, and marine toxins. Of these, perhaps the most important limiting factor in the maintenance of shellfish in a closed system is nitrogenous waste accumulation.<sup>47</sup>

The reaction of ozone with the various nitrogen compounds is not clear at this time. However, it is apparent that the end product of those reactions is nitrate. This is important because nitrates appear to be the least toxic with regard to shellfish activity with levels as high as 100 ppm exhibiting no effect on the metabolic processes.<sup>50</sup> The uncertainty as to the actual ozone reaction is due to the complex relationship between ozone and the characteristics of the water being treated; i.e., concentration of competing compounds, pH, temperature, etc. It has been found, however, that each of these parameters can have an optimizing effect on both disinfection and concentration reduction of the various seawater parameters.<sup>49,51-53</sup> This indicates the need for some degree of skill on the part of the operator in order to achieve proper results.

The use of ozone appears initially to be more costly than the use of a UV system with regard to operation. Power requirements tend to be higher depending upon the system used.  $^{53,54}$  However, the saving in water treatment cost which is anticipated for ozonation over ultraviolet light may more than offset the slightly higher operational cost of the ozone-unit.

Solids Disposal. The production of feces and pseudofeces by shellfish is a natural fact of metabolism. Because it may not be feasible to discharge those solid wastes into receiving waters under an NPDES permit, some form of removal followed by disposal may be required. The solids generated by depuration facilities can be disposed of through a number of techniques, including incineration, land filling, and land application. Srna, et al.<sup>55</sup> indicated that there were potential uses for those solids as a nutrient source for animal feed additives and fertilizers. However, that use may be limited by the quantity of gross, inorganic solids deposited in the system from the water source. In disposing of those solids various possibilities must be examined and the most economical technique applied to solve the problem.

### Economics

The literature provides no clear indication of depuration costs versus those for relaying. It does indicate that costs for each vary widely depending upon current prices, harvest yields, losses during processing, and processing time.<sup>9,23,56</sup> Holmsen and Stanislao<sup>56</sup> found that the estimated cost of depuration for quahogs in Rhode Island, using ultraviolet light, were approximately one-fifth that of transplanting. This evaluation did not examine the use of ozone, which as stated earlier would be slightly more expensive than a UV system, and did not consider pretreatment or posttreatment of depuration waters, a factor which may be more than offset by

ozonation. However, the cost adjustments would not be expected to change the economic justification.

Devlin<sup>23</sup> found that the estimated cost of oyster depuration in British Columbia was slightly higher than that for relaying. His analysis was also based on the use of ultraviolet light as a means of disinfection. However, his analysis did not consider losses in dual harvesting, total operational costs of equipment, harvest yield, or processing time with regard to relaying of the oysters. Two pilot plant depuration systems of one-bushel capacity were employed in this investigation. Those systems were constructed at the Oyster Biology Research facilities of the Gulf Coast Research Laboratory located at Point Cadet in Biloxi, Mississippi. They were constructed under a roof structure in order to eliminate exposure to direct sunlight and rainfall and consisted of a closed system and an open system. The two systems were placed in parallel. All functional units incorporated in the closed system were of identical geometry, volume, etc., as corresponding to units of the open system. A brief description of each pilot plant follows.

### Closed System

The closed system was constructed to provide for the recycling of water used in the depuration process. This system is illustrated in Figure 1. All of the tanks, with the exception of the depuration tank, were constructed of reinforced fiberglass. The depuration tank was made of exterior-grade plywood with the interior surfaces sealed with an epoxy resin. The joints of the depuration tank and the pipe connections were sealed with silicone rubber caulk. Piping consisted of either PVC or flexible plastic.

<u>Ozonation</u>. The tank used for ozonation of the depuration water had an approximate volume of 40 gallons (150 liters). Various sparger arrangements were used during the experiment in an attempt to promote maximum transfer of ozone to the depuration water. Compressed air was sequentially passed through a water trap, a ten micron filter, a dessicant, and a molecular sieve before it entered a Model C2P-3 PCI ozone generator.





The pressure on the inlet side of the generator was maintained between 18 and 24 pounds per square inch gauge (psig) while the outlet pressure was regulated between 10 and 15 psig. The ozone generation rate was generally set at 100 percent. This was necessary because early indications were that the rate of production of ozone at lower settings was not adequate to meet system requirements, including reaction with ammonia, organic and biological control of the depuration water. It should be noted at this point that at a 100 percent output the ozone supplied was not adequate to produce a depuration water free of all extraneous microorganisms. It was, however, sufficient to effect satisfactory reduction of coliform organisms.

<u>Aeration</u>. A single tank, having an approximate volume of 120 gallons (450 liters) and surface area of 6.3 square feet  $(0.58m^2)$  was used for ozonation and aeration. The tank was divided into two compartments, with the inner compartment used for ozonation, as is indicated by Figure 1. With this arrangement, approximately 80 gallons (300 liters) were allocated for aeration. The air was supplied from a blower which provided a line pressure of about two psig and was sparged through three aquarium-type glass stones.

Depuration. As previously noted, the depuration tank was made of exterior grade plywood. It was lined with an epoxy finish and sealed watertight. Internal measurements of the tank were four feet (120 cm) long, one foot (30 cm) wide and two feet (60 cm) deep on the inlet end. The bottom of the tank sloped 0.5 inches per foot (4.2 percent) toward the outlet end. The tank was fitted with two racks constructed of plasticcoated, one-inch wire mesh. Each rack was made to hold one-half bushel of oysters and was supported from the top of the tank by brass hooks. The racks measured 10 inches (25 cm) wide, 3.5 inches (9 cm) high and 27 inches (67 cm) long. The top rack was positioned six inches (15 cm)

directly in front of the inlet water entrance to the tank. This basket was parallel to and six inches below the top of the tank. The bottom rack was positioned at approximately six inches (15 cm) from the outlet port with a centerline depth of 18 inches (46 cm) from the top of the tank. Each basket had an empty weight of 2.38 pounds (1.1 kg).

<u>Settling Tank</u>. A settling tank, or clarifier, was used for separation of solids from the liquid during cleaning operation. This tank was a cylinder of four-foot (122 cm) height and 27-inch (69-centimeter) diameter with a conical end for sludge collection. The approximate volume of this tank was 120 gallons (450 liters) excluding the conical end. The influent to the tank passed through a five-gallon (19-liter), plastic container which served to dissipate the influent water's energy without disturbing settled solids and to equally distribute the flow laterally in the tank. (See Figure 1 for illustration.)

<u>Reservoir</u>. A reservoir containing water for recirculation throughout the closed system was required. A right cylinder, made of fiberglass, of approximately two-foot (60 cm) height and 3.4-foot (104 cm) diameter was used for this purpose. This arrangement provided an approximate volume of 120 gallons (450 liters) of water for recirculation, evaporation and sampling purposes.

<u>Pumps</u>. A Jabsco Model 17000 pump, powered by a 0.75-horsepower motor, was used for recirculation. This positive displacement pump had a flow rate in excess of the one gallon per minute desired for depuration. Flow regulation was achieved by using a system of valves whereby excess flow could be diverted back to the reservoir. Additionally, a small submersible pump was required between the depuration and settling tanks to facilitate cleaning of the depuration tank. This pump was placed in the line as indicated by Figure 1. This pump was used to hose down the

oysters in the trays as well as serving as a mechanism to transfer liquid from the depuration tank to the higher elevation of the clarifier.

## Open System

The open system was provided to compare operational parameters of a system identical to the closed system except that water used in depurating the oysters would not be recycled. This system is represented by Figure 2. All tanks, pumps, and piping were identical to those of the closed system with the exception of the pump reservoir at the head of the system. This was a fiberglass tank with an approximate volume of 1,000 gallons. An overflow standpipe was placed in the center of this circular tank to aid in maintaining a constant volume. Water was pumped directly from the Biloxi Bay to this tank and stored until needed.

Figure 3 contains photographs that represent various components of the system.








Figure 3(a): Depuration Train

Note from left to right:

- Ozonation/Aeration Unit
   Depuration Tank
   Clarifier
   Make-up Water Reservoir

Figure 3(b): Ozonation/Aeration Unit

Note the:

Circular Ozonation Tank
 Rectangular Aeration Tank



2.5

Figure 3(C): Depuration Tank Cleansing Process

Note: Closed-system clarifer in foreground contained water whereas the open-system clarifier was dry at the time of this photograph.



### Figure 3(d): Depuration Trays

Note: Tray positioned such that oysters diffuse influent waters at tank entrance. The same operational procedures and analytical methods were performed for both the closed and open depuration systems. Certain procedures were solely associated with changing oysters and, therefore, occurred once every three days while other procedures required daily performance. Finally there were operational procedures which were performed, as required, less frequently.

# Daily Operational Procedures

Each morning the exterior of the oysters and the depuration tank were cleaned of solids deposited during the previous 24 hours of operation. All of the solids obtained during this period were transferred to the clarifier through the inlet pipe. The procedure used was as follows:

- 1. The recirculation pump was shut off and the valve between the depuration tank and clarifier closed.
- 2. The bypass values which allowed pumping of the water contained in the depuration tank into the clarifier were opened and the water transferred.
- 3. The water level in the clarifier was lowered, using a bucket, to a point which allowed additions of depuration tank solids without solids loss to the weir.
- 4. The oysters and depuration tank were sprayed down, using a submersible pump in a bucket, and the solid/liquid mixture transferred to the clarifier.
- 5. The recirculation pump was restarted and the depuration tank refilled after closing the bypass values.
- 6. The valve between depuration and clarifier tanks was opened

only when the water level in the depuration tank was sufficient to prevent back flow from the clarifier.

7. The flow rate was checked and adjusted to a level between 1.0 gpm (3.8 lpm) and 1.2 gpm (4.5 lpm). That rate was also checked once or twice during the day to insure maintenance of that range.

The ozone generation system also required daily maintenance. This involved draining water from the air compressor tank and cleaning the water trap and the filter. Inasmuch as the water trap and the filter could only be cleaned when the air supply was shut off, the ozone generator was shut down prior to this procedure and turned on upon its completion. Finally the air pressure to and from the ozone generator was checked and adjusted to levels previously stated.

# Operational Procedures for Changing Oysters

The oysters were changed on a three-day cycle. After the depuration tank had been drained and the oysters and tank cleaned, depurated oysters would be changed to freshly harvested oysters. During this period the tank was scrubbed and lines to and from were taken apart and cleaned. Then the remaining daily operational procedures would be completed.

The oysters were harvested from areas which were known to be polluted. Some were harvested by dredge but the majority were collected by hand. The oysters were harvested within 24 hours of their placement in the depuration system. After harvesting and before being placed in the system, each oyster was thoroughly cleaned by hand using a gardentype hose with a sprayer attachment, then culled, and placed in the depuration trays. Before the trays were placed in the depuration tank, they were weighed and the weight and position recorded. After being depurated for approximately

1

three days, the trays of oysters were removed from the system and weighed, with the weight again being recorded. After weighing, the total number of oysters, as well as the number of dead oysters, was determined, and those values were also recorded.

#### Less Frequent Operational Procedures

Occasionally the water level in the reservoir of the closed system would reach a level which could permit air to be sucked into the pipes by the pump. At that same time the salinity would be at a maximum inasmuch as this loss of water would be due primarily to evaporation and sampling. In order to maintain the proper salinity and to prevent air being drawn in the lines, tap water with sodium chloride added was used periodically to fill the reservoir. The salinity of this make-up water was adjusted such that after equalization the salinity of the system's water would be equal to 15 parts per thousand (ppt).

Finally, sludge in the clarifier had to be withdrawn for measurement and evaluation. Unfortunately, it was impossible to remove the sludge accumulated in the clarifier without draining this tank because of the small quantity of solids produced during system operation. The water level was reduced using a submersible pump to a point where further reduction would have caused solids loss. The tank was then drained of the sludge using the same submersible pump after the solids were completely suspended in the remaining water.

#### Analytical Methods

Sampling of the water was performed for both systems in the same manner with the exception that one sample was taken from the closed system and two samples were taken from the open. Water samples constituted a grab sample taken from the clarifier effluent for both systems plus the ozone tank influent for the open system. A two-week intensive period of sampling and analysis followed the start-up of both systems after which the frequency of sampling was reduced only to days when the oysters were changed. Sludge samples were collected from both systems at the end of operation. In addition an intermediate sludge sample was taken from the closed system.

A number of analyses were performed on the water samples which insured proper operation of the depuration process and indicated the degree of degradation of the depuration waters and requirements for subsequent wastewater treatment. Operational parameters used in this investigation follow.

<u>Salinity</u>. Salinity was measured using an American Optical Goldberg refractometer. In the closed system the salinity was maintained between 15 and 16 ppt. As indicated earlier, this was accomplished through the addition of artificially saline tap water. No attempt was made to control salinity in the case of the open system.

<u>Turbidity</u>. Turbidity was determined using a Hach turbidimeter, Model 2100A. Turbidity was measured as Jackson Turbidity Units (JTU's). The only attempt to control turbidity was incorporated in the design of the depuration systems through gravitation means.

<u>Dissolved Oxygen</u>. Dissolved oxygen was measured <u>in situ</u> using a YSI Model 54 oxygen meter and probe. The meter was standardized using the azide modification of the Winkler method as presented by <u>Standard Methods</u>,<sup>57</sup> Part 422B. The influent and effluent concentrations of dissolved oxygen were measured in the depuration tank to insure that sufficient oxygen existed to support the oysters. Occasionally a profile of the dissolved oxygen level throughout the depuration tank was determined to insure that

no section exhibited low oxygen levels.

The discharge limitations set forth in NPDES permits control the level of permissible water degradation. Several components can make up this permit. Those examined in this investigation follow.

<u>Suspended Solids</u>. The procedures outlined in <u>Standard Methods</u>,<sup>57</sup> Parts 208D and E, were used in determining the suspended solids concentration of the depuration waters. Those procedures were used to determined the amount of total, volatile, and fixed suspended solids.

<u>Biochemical Oxygen Demand (BOD5</u>). Filtered and unfiltered BOD5 were determined using the method outlined in Part 507 of <u>Standard Methods</u>.<sup>57</sup> Dilution water was made by adding sodium chloride to distilled water in concentrations to yield a salinity of 15 ppt. Seed was used in all samples and was made by aerating filtered seawater for a minimum of 24 hours. The method for calculating the BOD5 of the water is outlined in Appendix A.

<u>Total Kjeldahl Nitrogen (TKN)</u>. A modification of the Kjeldahl procedure outlined in <u>Standard Methods</u>,<sup>57</sup> Part 421, was used in this investigation. This modification, eliminating the distillation step, and using an ammonia probe for TKN determination, is outlined in Appendix B. Both filtered and unfiltered TKN were determined for the samples collected.

<u>Nitrate Nitrogen (NO<sub>2</sub> - N)</u>. The method used for this determination is presented by Strickland and Parsons<sup>58</sup> and involves the diazotizing of filtered nitrate sample to form an azo dye. The samples were read colorimetrically using a Coleman 124D, ultraviolet-visible spectrophotometer. This method is very similar to that presented by EPA.<sup>59</sup>

<u>Nitrate Nitrogen (NO<sub>3</sub> - N)</u>. The filtered nitrate sample was passed through a cadium-copper column reducing the nitrates to nitrites. Nitrites were determined as indicated above. The reduction column used was as presented by Strickland and Parsons.<sup>58</sup>

<u>Total Phosphorus (P)</u>. Total phosphorus was determined in accordance with the method published by EPA<sup>59</sup> using the persulfate digestion procedure. The samples tested were unfiltered and filtered.

The final component examined in this investigation was that of the sludge generated by the depuration systems. The quantity of sludge was measured and suspended solids were determined in accordance with the procedures outlined above for the water samples. In addition to these determinations the following analyses were performed on the depuration sludge.

<u>Oxygen Consumption Rate</u>. The oxygen consumption rate of the sludge was measured using the method outlined in <u>Standard Methods</u>,<sup>57</sup> Part 213B. A BOD bottle was used in conjunction with a DO meter and probe. The sludge sample was aerated for an extended period, placed in the BOD bottle, and the level of dissolved oxygen measured with time.

<u>Oxygen Transfer</u>. As indicated in <u>Standard Methods</u>,<sup>57</sup> Part 207B, there are three components which need to be determined with regard to the transfer of oxygen into a waste; i.e., the uptake rate, r, the ratio of K<sub>L</sub>a for wastewater to that of clean water, alpha, and the ratio of saturation concentration for the wastewater to that of clean water, beta. Each of these was determined as outlined in Part 207B using a dissolved oxygen meter and probe. In the case of alpha determination a 500ml sample was placed in a beaker and stirred rapidly using a magnetic stirring device. Care was taken during the measurements to prevent direct contact between the atmospheric air and oxygen probe's membrane. The other tests were performed using a BOD bottle as opposed to a beaker.

<u>Settleable Solids</u>. The method used in determination of the settleable solids content of the sludge is presented in Part 208F of Standard Meth-<u>ods</u>.<sup>57</sup> This volumetric test indicates the gross quantity of easily

settleable solids.

<u>Zone Settling Rate</u>. An important component in the design of sedimentation tanks, the zone settling rate of this sludge was determined using the procedure outlined in Part 213D of <u>Standard Methods</u>.<sup>57</sup> A graduated cylinder was used in this determination. The sludge was applied rapidly at the start of measurement, as opposed to using a mechanical stirring device, to maintain suspension of solids until measurement was begun.

Both water and sludge samples were collected in one-gallon, plastic containers. It should be noted that samples were rapidly cooled to 4°C if any short-term storage was required before testing. However, in the case of nitrite and nitrate samples and total phosphorus samples, longer storage was required. The nitrogen samples were filtered, placed in plastic bags and frozen while the filtered and unfiltered phosphorus samples were placed in plastic bags, acidified with sulfuric acid, and frozen. At the outset of this project, a detailed dye study was performed on the closed depuration system. The purpose of that study was to observe flow patterns and model hydraulic retention time in various components of the system. Because of the symmetry of the two systems, a test was not made on the open system. The test was performed with the depuration trays in place and filled with clean oyster shells.

The results of that test are presented in Figure 4. That illustration indicates that the water was retained in the ozone and aeration tanks for a period of approximately 90 minutes. Due to the arrangement of those two tanks (see Fig. 1) it was not possible to determine retention in each tank separately. Other observed retention times were 60 minutes in the depuration tank and 75 minutes in the clarifier. The observed retention time in the depuration tank was as expected. The hydraulic retention time provided by the clarifier indicates that there was a volume of approximately 45 gal. (210 1) in the lower section available for sludge storage.

### Closed System

The average weight of one bushel of oysters used in the closed system was 70.5 lb (32.0 kg). Each bushel experienced a slight reduction in weight during the three-day depuration period. In the case of the closed system that reduction was 0.3 lb (0.1 kg); the final average weight was 70.2 lb per bushel of oysters (31.9 kg/bu). That loss in weight can be explained in part by oyster metabolism and mortality. Of the average 297.2 oysters per bushel placed in the closed system, an average of 3.1 oysters died giving a mortality rate of approximately one percent during the three days of depuration.



(%) əənsdrosdA

<u>Biochemical Oxygen Demand</u>. The biochemical oxygen demand, BOD5, of the water contained in the closed system and its variation during operation of the system are presented in Figure 5. Because of the scatter of data points, a least squares regression was performed to determine the equations which best represent the trend exhibited by BOD5 over an extended period of time. Those equations were:

$$BOD_u(t) = 2.04 + 0.01t$$
 (1)

and

$$BOD_f(t) = 1.08 + 0.01t$$
 (2)

where  $BOD_u(t)$  and  $BOD_f(t)$  equal the unfiltered and filtered biochemical oxygen demand respectively, in mg/l, after t days of system operation. From those equations one can observe that there was a slight, gradual degradation in the BOD5 of the water in the closed system with respect to operation and that the degree of degradation was directly dependent on the operation time. Those depuration facilities that utilize waters from Mississippi Gulf coast estuaries may be required to operate in a closed system mode for as long as 60 days because of problems associated with low salinity and high turbidity. If equations 1 and 2 are used to determine the level of BOD5 after 60 days of operation, the unfiltered and filtered BOD5 of the waters in the closed system operation would be 2.5 and 1.8 mg/l, respectively. Those BOD5's are indicative of only a slight degradation of depuration waters in a closed system, yielding an increase of approximately 1.5 mg/l BOD5 after 60 days operation. Daily variation in the quality of estuarine waters should be greater than that observed in the depuration facility.

There should be little or no need to provide additional treatment of waste waters from closed systems as long as ozone is used as the disinfectant to control biological activity in depuration waters. Should UV be





used as the disinfectant, depuration waters may used as the disinfectant, depuration waters may degradate to a level that would require removal of water soluble metabolites before being discharged into a receiving stream. Although data are not available to say this requirement would be absolute, Figure 5 seems to indicate that would be so.

<u>Suspended Solids</u>. The suspended solids in wastewater that is to be discharged is another important parameter. Variations in suspended solids with respect to time in the closed system's operation are presented in Figure 6. A least squares regression analysis and other methods of curvefitting were employed in an attempt to develop expressions defining trends in water quality. Expressions were developed similar to Equations 1 and 2; however, they were of little value in estimating trends because of the extreme variability of the data.

An "estimated" smooth curve was imposed on Figure 6. An inspection of the data indicates a very definite improvement in the quality of depuration waters over an extended period of time. It may be anticipated that, after an extended period of operation, total suspended solids in a closed system would be less than 2 mg/l. Since this concentration is less than that found in natural estuarine environments along the Gulf Coast, removal of suspended solids beyond what is achieved in a clarifier should not be required prior to discharge. It should again be noted that waste water produced in daily cleansing procedures of the depuration tanks was directed to the clarifier where excess suspended solids were effectively separated from the water.

Again, insufficient data were taken after removal of the ozone sparger from the system to draw any conclusions as to ozone's effect on the concentration of suspended solids. Little effect would be expected with regard to





fixed suspended solids, but volatile suspended solids, and thereby total suspended solids, may very well increase. A preliminary indication of that possible increase was the turbidity of the system's water. That parameter is presented in Figure 7. In that figure a value of 1 JTU was used if the value was less than or equal to 1 JTU. That figure indicates that relatively rapid reduction in turbidity occurred during normal operation of the system and that upon removal of the ozone sparger the turbidity of the waters began to increase. The similarity between Figures 6 and 7 implies that an increase in suspended solid concentration might be expected after cessation of ozonation. Similarly that increase in solids could cause an increase in the BOD<sub>5</sub> of the water.

<u>Total Kjeldahl Nitrogen</u>. The total Kjeldahl nitrogen, TKN, represents a combination of organic and ammonia nitrogen, both of which are by-products of biological metabolism. The TKN of the depuration waters in the closed system and its variation during system operation are presented in Figure 8. As before, the least squares regression method was employed to define lines representing these results. The equations that best fit those data are:

$$TKN_{u}(t) = 1.96e^{0.01t}$$
 (3)

and

$$TKN_{f}(t) = 1.56e^{0.01t}$$
 (4)

where TKN<sub>u</sub>(t) and TKN<sub>f</sub>(t) equal the unfiltered and filtered total Kjeldahl nitrogen respectively, in mg/l, after t days of system operation when ozone is used for disinfection. Equations 3 and 4 indicate that the slight degradation of the system's water with respect to TKN is a gradually diminishing function. They additionally implied that this increase in concentration was due primarily to soluble TKN because the ratio of unfiltered to filtered TKN was essentially constant. If we apply those equations to



(UTL) YaibidauT



Total Kjeldahl Nitrogen, (mg/l)

determine the degree of degradation in a 60-day period, values of 3.6 and 2.8 mg/l are obtained for unfiltered and filtered TKN, respectively. This represents an increase of only 1.6 mg/l unfiltered TKN over 60 days of operation.

It was indicated earlier that the ozonation of the depuration waters was, at best, marginal because the transfer of ozone from the gas phase to the liquid phase was not as good as could be expected. A well-designed ozonation system may reduce TKN to a level below that predicted by Equations 3 and 4.

Figure 8 illustrates the effect of ozonation on controlling the system's TKN. A marked increase in both unfiltered and filtered TKN is illustrated in Figure 8 and Equations 5 and 6, which where determined by least squares regression for the data included in that section where ozonation was discontinued:

$$TKN_{1}(t) = 0.27t + 1.94$$
(5)

and

$$TKN_{x}(t) = 0.16t + 1.58$$

If we apply those equations to a 60-day operating period, the final values for unfiltered and filtered TKN would be 17.9 mg/l and 11.3 mg/l, respectively. Those results indicate that some method of nitrification of the system's waters would probably be required prior to discharge into a receiving water if ozonation is not incorporated into the depuration system.

<u>Nitrites and Nitrates</u>. Nitrites and nitrates are not measured by the TKN analysis. They are, however, important when an estimate of total nitrogen in wastewater is required. Figure 9 illustrates the nitrite and nitrate concentrations in the effluent from the clarifier over an extended period of time. Since a correlation of the data was not statistically significant only general statements will be made. An inspection of Figure 9

(6)



Nitrite and Nitrate Concentrations, (mg/l)

indicates that nitrites are easily and effectively oxidized to nitrates in the presence of ozone. On the other hand there was not an apparent buildup of nitrates in the system during 40 days of depuration. Data were not available in sufficient quantity to estimate build-up of either nitrates or nitrites for periods of time in excess of 40 days.

<u>Total Phosphorus</u>. The total phosphorus concentration in the effluent from the clarifier was studied over an extended period of time. Results of that study are presented in Figure 10. An inspection of Figure 10 indicates that most of the total phosphorus was in a soluble form. Very little appeared to be organically bound to particles of detritus or to the bodies of aquatic organisms.

The build-up of phosphorus within the closed system may be attributed, in part, to the effects of evaporation of process waters from the system and the subsequent addition of make-up water. Requirements for make-up water will be discussed later in this report.

<u>Sludge Generation</u>. Other important criteria in the design, installation and operation of a system of this nature are the quantity and quality of sludge generated during depuration. The quantity of sludge has a direct bearing on treatment unit sizing, while the quality determines the treatment methods that may be employed. During the 44 days of operation of this one-bushel depuration system, 7.9 gal (30 1) of sludge were collected in the clarifier. That approximates 0.18 gal (0.7 1) of sludge per day per bushel of depurated oysters.

Sludge was collected twice during the depuration experiment; once after 19 days and then again at the end of 44 days. Sludge characterization was performed on both samples. It should be noted that no floating solids were observed prior to the first sludge collection although floating solids were found after an additional 23 days of system operation. Therefore, if



Figure 10: Total Phosphorus Versus Time - Closed Depuration System

sludge is to be periodically withdrawn from a system of that type, that removal should occur as frequently as possible. Since a 100-bushel facility would produce approximately 18 gal (68 1) of sludge daily, it would not be practical to withdraw sludge more frequently than daily. Depending upon local operating conditions, less frequent sludge withdrawal, such as once every two or three days, may be preferable to daily removal.

<u>Settleable Solids</u>. An analysis of settleable solids in the sludges indicated that approximately 1.3 gal (5 1) of solids were generated during 44 days of operation. The daily average was 0.03 gal (0.1 1) of settleable solids per bushel. In addition, the average content of total suspended solids (TSS) of the sludge collected during this 44-day period was 172 gm. Thus, the TSS averaged 3.9 gm ( $8.6 \times 10^{-3}$  1b) per day per bushel of oysters.

Other analyses were performed on those sludge samples as indicated previously. The results of those tests are presented in Table 1. It is apparent from the data that those factors which are important in the design of aerobic biological treatment systems are highly variable. That variability may be explained, in part, by the different techniques used to remove the sludge from the clarifier. The 19-day sample of sludge was removed while the system was in operation and the 44-day sample was collected after the clarifier was drained. It was impossible during the first withdrawal to remove all of the sludge; some remained in the clarifier until the final cleansing. During that time additional biological stabilization of the sludge occurred, causing a decrease in both the oxygen consumption rate and the oxygen uptake rate.

If aerobic stabilization is used for sludge treatment, the higher values from Table 1 should be selected. However, because of the extremely small volume of sludge generated and the high salinity of the water, sludge treatment by some other means would be preferable.

## TABLE 1

## Characterization of Sludge Samples From Closed Oyster Depuration System Clarifier

CHARACTERISTIC	SLUDGE FROM	
	FIRST 19 DAYS	LAST 23 DAYS
Oxygen Consumption Rate, (mg/gm/hr)	7.4	1.2
Oxygen Uptake Rate, r, (mg/1/hr)	7.2	3.0
Alpha, a	1.0	0.1
Beta <sup>(1)</sup> , $\beta$	0.9	1.0
Zone Settling Rate, (ft/min)		0.24

(1) Adjusted for Salinity.

The results of the zone settling analysis indicated a rather rapid solid/liquid separation. Gravity thickening of sludges from the clarifier may be achieved with a relatively high degree of efficiency. Again, because of the small volume of sludge produced, it would be difficult to justify including gravity thickness in the design of waste treatment facilities.

Make-up Water. The final consideration in the operation of the closed, oyster-depuration system was the quantity of water required to replace that lost through evaporation, leakage, etc. It was found, after taking sampling losses into account, that during 42 days of operation approximately 400 gal (1,500 1) of water were required. The average daily use was 9.5 gal (36 1). The rate of evaporation is directly related to the exposed water surface area. For the pilot-plant system used in this investigation, that area was 23.3 ft<sup>2</sup> (1.9 m<sup>2</sup>). Therefore, approximately 0.41 gal was required per day of operation per square foot of water surface area (16.7 1/day/m<sup>2</sup>). That was also 0.66 in/day (1.67 cm/day) or 19.7 in/month (50 cm/month). That was much higher than the average evaporation rate that was expected. For example, the average, natural evaporation rate from the three highest months for the ten most recent years at the LSU Ben-Hur Experimental Station was 7.4 in (19 cm) per month. <sup>60</sup> That was the closest weather station reporting evaporation data and it pointed out that other factors play a significant role in the quantity of make-up water required.

#### Open System

The average weight of one bushel of oysters placed in the open depuration system was 71.7 lb (32.6 kg). As in the closed system each bushel experienced an average weight reduction of 0.3 lb (0.1 kg/bu) and the final, average bushel weight was 71.4 lb (32.5 kg). Each bushel placed in the open system contained an average of 330.7 oysters. An average of 3.5 oysters died during the three-day depuration period thereby resulting in a mortality rate of approximately one percent.

<u>Biochemical Oxygen Demand</u>. The BOD5 data for the influent and effluent samples of the open system are presented in Figure 11. That figure indicates that the effluent BOD5 of the open system can be expected to be slightly higher than that of the influent. It also indicates that the slight degradation in water quality expected in such a system is probably not sufficient to warrant treatment of the effluent prior to discharge. An attempt to linearize these data, and the results which follow, proved relatively fruitless other than to confirm that which was visually obvious. However, the degree of water degradation illustrated by Figure 11 does confirm that without ozonation of the waters the rate of degradation with respect to this parameter would be greater than the slight deterioration predicted by Equations 1 and 2 for the closed system.

<u>Suspended Solids</u>. Figure 12 illustrates the concentration of suspended solids in the waters influent and effluent of the open system. As in the case of the closed system, that figure indicates that an improvement in water quality with respect to suspended solids can be expected. It also indicates that a large percentage of the non-volatile solids will remain within the depuration system and that large variations in the concentration of influent solids will not be exhibited by the effluent concentration; i.e., the concentration of suspended solids in the effluent will remain relatively constant when compared to variation in the influent quality. That fact again tends to confirm the observations from the closed system.

<u>Turbidity</u>. A further indication of the concentration stability of effluent solids is turbidity. The influent and effluent values of this parameter are represented in Figure 13. That figure illustrates that the



Biochemical Oxygen Demand, (mg/l)





Total and Volatile Suspended Solids versus Time -Open Depuration System

Figure 12:



effluent turbidity of the open system was only slightly dependent on variations in the influent turbidity. It further indicates that turbidity can be expected to be reduced to a significant degree by this system. Because of those facts and the conclusions drawn from Figure 12, it appears that treatment for solids of the effluent prior to discharge should not be required.

<u>Total Kjeldahl Nitrogen</u>. Figure 14 presents the total Kjeldahl nitrogen data obtained for the open system and indicates that a slight degradation of the water passing through this system can be expected. That figure also confirms the basic premises developed for the closed system, that the degree of degradation tends to increase with operation time and that without ozonation the rate of deterioration will be greater. However, Figure 14 implies that the level of degradation in all probability would not be sufficient for an open system to warrant treatment of the effluent prior to discharge.

<u>Nitrite and Nitrate</u>. The results of the nitrite and nitrate analyses of the influent and effluent waters of the open system are presented in Figure 15 and 16, respectively. Those figures reveal that the concentration of each component is relatively low for both the influent and the effluent waters. However, little else can be determined. The nitrite data do imply that if degradation occurs within the system, it is negligible. In fact, the nitrite concentration decreased very slightly in the closed system. Additionally, nitrate appears to increase in the closed system; however, that cannot be confirmed upon examination of the data from the open system. In any case, denitrification of the open system effluent should not be required before discharge.

Total Phosphorus. The unfiltered total phosphorus concentrations of both the influent and effluent of the open system are presented in Figure 17.



Total Kjeldahl Nitrogen, (mg/l)



Nitrite Concentration, (mg/l)







Total Phosphorus Concentration, (mg/l)

One should note the slight increase of total phosphorus in the unfiltered effluent from an open system. That increase in phosphorus is so slight, and the concentrations are so low, that it would be difficult to determine with a high degree of certainty whether the degradation is real or apparent.

Sludge Generation. The final considerations in the operation of an open system were the quantity and quality of sludge produced. During 23 days of operation, approximately 6.6 gal (25 1) of sludge collected in the clarifier while approximately 1.1 gal (4 1) of sludge accumulated in the aeration tank. An average 0.29 gal (1.1 1) of sludge was deposited in the clarifier and 0.05 gal (0.17 1) of sludge was deposited in the aeration tank per day per bushel of oysters. Although no analysis of the sludge from the aeration tank was performed nor were any volumetric measurement made of the solids accumulation in the pump reservoir, those solids would have to be contended with if a surface water is used as a source for depuration process water. The same analyses performed on the sludge from the closed system were used to characterize sludge from the clarifier of the open system. Floating solids were first observed in the clarifier on the 22nd day of operation, thereby confirming the sludge removal procedure discussed previously for the closed system.

Settleable solids. Settleable solids analysis of the sludge indicated that approximately 0.58 gal (2.2 1) of solids were generated during the 23 days of operation. A daily average of 0.03 gal (0.1 1) of settleable solids were generated per bushel of oysters. That correlates closely with the daily average of the closed system. However, the average amount of total suspended solids (TSS) in that sludge was 207 gm or 9.0 gm (2.0 x  $10^{-2}$  lb) of TSS per day per bushel of oysters. This implied that the sludge from the open system was more concentrated and compressible than that of the closed system and, therefore, contained additional solids due

to the content of solids in the influent.

The results of other analyses performed on the sludge are presented in Table 2. One may note that oxygen requirement for stabilization of sludge from the open system was approximately twice that for the closed system. That was due, in part, to the fact that sludge from the open system was much more concentrated.

Because of the very small volume of sludge generated and the variability in oxygen requirements, aerobic methods of sludge stabilization would not be required for treatment purposes.
# TABLE 2

## Characterization of Sludge Sample From Open Oyster-Depuration System Clarifier

Characteristics	Value
Oxygen Consumption Rate, (mg/g/hr)	14.0
Oxygen Uptake Rate, r (mg/l/hr)	13.2
ALPHA, a	0.07
BETA <sup>(1)</sup> , $\beta$	1.0
Zone Settling Rate, (ft/min)	0.13

(1) Adjusted for Salinity

...

This investigation examined, in detail, the effects of oyster depuration on waters in both flow-through (open) and recirculating (closed) pilot plant facilities of symmetrical design. Not only did these experiments indicate the degree of water degradation but they also provided insight into the degree of simplicity required for the operation of a depuration facility.

# Operational Simplicity

The labor requirement for operating a depuration facility was relatively low. After the basic operational parameters were established in the system, the process proceeded with a minimum of attention being required for proper operation. Periodic monitoring of flow rate and general inspection of system components to insure proper operation were required. Operation of a full-scale facility should not increase, and may even decrease, labor requirements for this purpose because minor variation in system parameters would not have as significant effect on operation as they did in the pilot plant facility. Furthermore, sophisticated control devices are more easily incorporated in a larger system thereby permitting automatic correction of critical hydraulic parameters.

The highest labor requirement in the daily operation of the depuration systems was for routine cleaning of oysters both before and during depuration. The oysters used in this investigation were culled and cleaned by hand to remove excess solids from the shell surface before being placed in the system. That process can easily be automated in a full-scale facility and would virtually eliminate manual involvements.

It should be pointed out at this time that although we did not attempt

to characterize wastes from pre-depuration cleansing, those wastewaters would require significant disposal attention. The treatment of those wastes could be readily incorporated into the disposed technique for the solids generated in the depuration system.

Daily cleaning of the oysters in the depuration system required the cessation of operation for all facility components. That allowed not only transfer of solids from the oysters and depuration tank to the clarifier but preventive maintenance of the facility components. That period, during which depurated oysters were removed and replaced with those requiring depuration, was the most labor intensive period of the operational process. That should also be the case in the operation of full-scale facilities. However, during the operation of the pilot plant, proper planning of required work prior to the shut-down procedure greatly minimized time requirements, thereby reducing the labor requirements and system down-time. The same should apply to full-scale facility operation.

### Depuration Water Degradation

The results presented in this report indicated that water degradation in both open and closed systems was not significant with regard to acceptable levels for discharge into receiving waters. Two system components deserved the credit for the maintenance of depuration water quality. Those components were the sedimentation tank(s) and the ozone disinfection system.

<u>Sedimentation</u>. A clarifier was included in the pilot plant facilities immediately downstream from the depuration tank. That unit removed solids produced during the depuration process, thereby limiting the degradation of process water to non-settleable components. Although the quantity of solids collected during these experiments was not significant, the

presence of those solids in discharged waters would create a significant problem in receiving waters with respect to the parameters measured and solids deposition. That is the justification for including the limitations on solids in NPDES permits and the removal of the solids generated during depuration would be required under such permits.

As previously stated, the open system that was examined during this investigation contained a constant-head reservoir from which water was pumped into the system. This reservoir served a dual function in that it also removed gross solids contained in the plant influent. Although solids deposited in that tank were not examined for content or volume, it should be noted that they were significant in quantity.

Again, the requirement for such a tank in a full-scale system is dependent upon the water source. If such a requirement exists, it should be carefully designed. The importance will be determined by the quantity of sludge deposited in the aeration tank of the pilot plant. Whereas the solids deposited in that unit appeared similar to those deposited in the reservoir, variations in flow rate effected the reservoir loading rate and, hence, the efficiency of solids removal.

Ozone Treatment. The available literature indicates that ozone is an extremely powerful oxident when used to eliminate bacteria and virus. The efficiency of this technique rivals ultraviolet irradiation of waters and surpasses it with regard to the effects of other contaminants on the efficiency of disinfection. However, on a purely disinfectant basis, the use of ozone is more expensive than the use of ultraviolet light. On the other hand, the results of this investigation confirmed the benefit of ozonation which may more than off-set the additional cost. That benefit is the maintenance of water quality.

In the operation of a closed system ozone oxidized most of the soluble

and suspended metabolites produced by the oysters. Therefore, the biochemical oxygen demand, suspended solids concentration, and TKN of the water are reduced to some extent and the rate of water degradation is retarded. That fact is supported in the literature and was most evident in the rate of TKN degradation with and without ozonation in the closed system's waters. It was also confirmed by the nitrite and nitrate data which were by-products of TKN oxidation. Nitrites, which are readily oxidized to nitrates, were present at low concentrations while the concentration of nitrates increased.

The phosphorus data from our investigation are strange but do tend to support the oxidation premise in that phosphorus is a minor component of feces and psuedofeces. Further, the phosphate form of phosphorus is an oxidized state and would not be affected by further oxidation. Therefore, the concentration of total phosphorus in the water would not be reduced by ozonation as were the concentrations of BOD5 and TKN. However, its rate of increase was much greater than was expected from the simple oxidation of organics. It therefore appears that some other component such as makeup water and concentration through evaporation could be the cause. Unfortunately, the phosphorus content of make-up waters was not determined.

In the open system, we expected that the levels of pollutants in the influent to be reduced to some extent by ozonation, thereby reducing the level of those same components in the effluent. That might further explain the deposition of solids in the aeration tank because oxidation would tend to stabilize those suspended components thereby allowing their removal by gravimetric means. Additionally, that technique could be applied to the effluent in order to reduce the level of pollutants as required by an NPDES permit.

### Summary

The depuration process which we examined constitutes a significant means for the purification of shellfish for human consumption. This investigation provided evidence that:

- The quantity of solids generated in the depuration process which can be readily removed by conventional gravimetric technique(s);
- The use of ozonation in the depuration facility provided adequate disinfection and reduced the degradation of the process water;
- 3. The effects of ozonation will reduce, or eliminate, the need for water treatment prior to discharge which may in turn offset the higher operational cost of ozonation compared to ultraviolet irradiation; and,
  - 4. A closed depuration system can be operated for an extended period without significant problems and that same system will function adequately in an open mode of operation.

### VII. RECOMMENDATIONS

This study addressed problems associated with the degradation of process water used for depurating oysters in open and closed systems. In some instances the quality of water within the system had a noticeable improvement, eg. the decrease of suspended solids. When degradation occurred, as typified by a very gradual increase of BOD5, it was limited. However, there are several recommendations as a result of this study that should be considered when implementing commercial depuration in the future.

### Ozone or UV-Light

We recommend that ozone be utilized as a disinfectant in the <u>closed</u> <u>system</u> depuration process, or for open systems that have significant problems with turbidity. The results of this study indicate that there may be significant problems associated with degradation of water quality in the absence of ozone. It should be noted that it was not the purpose of this study to compare the merits of both methods of controlling biological and chemical quality of depuration process water. The results of this study are insufficient to positively demonstrate that the quality of the depuration water will be degraded if UV-light is used. It is clear, however, that degradation is minimal through 44 days of operation of a closed system when ozone is used.

Based on observations from this study, we also recommend that additional water degradation studies be conducted if UV-light is to be used for water quality control in closed depuration facilities. If degradation does occur, the additional costs of installing ozone generation equipment would be more than off-set by treatment costs for wastewater prior to discharge.

### Preparatory Cleaning

Unprocessed oysters may have significant quantities of sediment and other foreign matter attached to exposed surfaces that must be removed before depuration. Those materials should be removed at the time the animals are harvested because of the difficulty of removing them after they have dried or consolidated.

Cleansing of the shellfish may be accomplished by either highpressure spraying or by mechanical brushing. We recommended that high-pressure spraying be used in preference to mechanical brushing because of the reduced maintenance associated with spraying. Water used for this purpose may be either fresh or saline.

Wastewaters generated during the shell-cleaning process will contain significant quantities of suspended solids. Those solids can be easily separated in a clarifier or by filtration. If fresh water is used for the preparatory cleaning, sedimentation, followed by filtration, would produce an effluent which satisfies discharge standards for sewers or receiving streams. On the other hand, saline waters would be sufficiently treated for solid/liquid separation in the same clarifier used for the control of solids produced during the depuration process itself.

The transfer of coliform bacteria and other undesirable microorganisms from the exterior shell to the depuration tank will be reduced by providing a five-minute contact with a solution containing 5 to 10 mg/l of free, available chlorine. That could be accomplished after removal of foreign matter from the shells by placing the shellfish in depuration trays and immersing them in the solution. It is essential that all chlorine be flushed from the shellfish and baskets before transferal to the depuration tanks.

### Process Wastes

The major water-quality contaminants in depuration process water are feces and pseudofeces produced by the shellfish. Those contaminants are easily removed as suspended or settleable solids. We recommend that wastewaters produced during routine tank cleaning procedures be processed through a clarifier for solid/liquid separation before being recycled in a closed system, or before being discharged from an open system.

The process water from a closed system would be discharged, after an extended period of operation, and replenished with a fresh supply. Ozonation (or chlorination) must be provided before the process water is discharged from a closed system into receiving waters for reduction of coliform. We recommend that chlorination be used in conjunction with UV-light disinfection of process water. The additional ozone requirements would be very small for systems operating with ozone disinfection of process waters.

Other parameters such as BOD5, TKN, total phosphorus, etc., do not appear to significantly deteriorate the quality of process water over an extended period of closed system depuration. Because the results of this study can only be discussed as slight trends over a finite time interval, we recommended that additional wastewater treatment not be provided for proto-type facilities to be constructed along the Mississippi Gulf coast. We recognized that there are coastal areas of Mississippi and elsewhere where additional treatment of process wastewater may be required. We recommended that sites for depuration facilities be selected, and then regulatory agencies be approached for treatment requirements at that site.

### Sludge Solids

The sludge from the clarifier used for solid/liquid separation will putrify and become very offensive upon removal. Aerobic sludge stabilization is not recommended because of the very small volumes generated. When local conditions permit, digestion of this sludge may be accomplished in a properly designed and operated septic tank and field sorption system. Pumpage from the septic tank would then be transported to a sanitary landfill for final disposal.

An alternative would be to stabilize the sludge by chemical means. Chemical stabilization may be accomplished by adjusting the pH to 11.0 units and holding for land application or landfilling. Chemical stabilization of sludge is recommended for areas where septic tank systems are not permitted.

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# X. APPENDICES

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# APPENDIX A

### (BOD)

Use 2 ml seed per liter of dilution water.

$$mg/1 BOD = \frac{(D_1 - D_2) - (B_1 - B_2) f}{P}$$

Where:  $D_1 = D0$  of diluted sample 15 minutes after preparation  $D_2 = D0$  of diluted sample after 5-day 20°C incubation period  $B_1 = D0$  of dilution of seed control before incubation  $B_2 = D0$  of dilution of seed control after 5-day 20°C incubation f = ratio of seed in sample to seed in control  $= \frac{\chi \text{ seed in } D_1}{\chi \text{ seed in } B_1}$ P = decimal fraction of sample used

Other Symbols Used

$$\Delta D = (D_1 - D_2)$$
  

$$\Delta B = (B_2 - B_1)$$
  

$$U = Unfiltered BOD$$
  

$$F = Filtered BOD$$

Volume of BOD bottles = 300 ml

Seed correction =  $(B_1 - B_2)$  f

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APPENDIX B

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### (Micro)

The following references must be consulted in conjunction with the following:

- a) Instruction Manual: Ammonia Electrode, Model 95-10 by Orion Research
- b) Standard Methods for the Examination of Water and wastewater. 14th Ed., APHA-AWWA-WPCF, Washington, D. C. (1926).

All glassware must be thoroughly clean. Water used for cleaning glassware, preparing reagents, and diluting samples, etc., must be ammonia free.

### Equipment

LABCONCO Digestion Apparatus or equal

Digestion flasks - 100-ml capacity

Orion Ammonia electrode, Model 95-10

Orion Specific Ion Meter, Model 407

### Apparatus

Adjust the temperature controls on the digestion apparatus so that 50 ml of distilled water at an initial temperature of 25°C can be heated to a rolling boil in approximately 5 minutes. The thermostat for each heater must be individually adjusted.

### Reagents

### **Digestion Reagent:**

Dissolve 134 gm potassium sulfate,  $K_2SO_4$ , in 630 ml ammonia-free distilled water and 200 ml concentrated  $H_2SO_4$ . Add, with stirring, a solution prepared by dissolving 2 gm red mercuric oxide, HgO, in 25 ml 6N  $H_2SO_4$ . Dilute the combined solution to 1 l. Store this solution at a temperature above 14°C to prevent crystallization.

### Alkaline Reagent:

400 gm sodium hydroxide

332 gm potassium iodide dissolved and diluted to one 1

Ammonia free Water:

See Standard Methods, pages 5 and 410

## Standards:

See Instruction Manual, page 5.

Prepare a calibration curve; Instruction Manual, page 6.

### Procedure

This procedure is similar to <u>Instruction Manual</u>, page 17.

- 1. Add 5 boiling beads to a clean, 100-ml, Kjeldahl flask.
- 2. Add 50 ml of sample (or sample and enough distilled H<sub>2</sub>O to 50 ml).
- 3. Add 10 ml of digestion reagent.
- Digest the sample. Continue digestion for 0.5 hr after SO<sub>3</sub> is evolved.
- 5. Cool to room temperature or lower.
- 6. Dilute to 100 ml volume with Ammonia free water in a graduated flask.
- Take dilutent and proceed using the Ammonia probe with <u>alkaline</u> reagent as a caustic. (Not NaOH)
- 8. Run blanks and standards in this same manner. Observe the same time interval between Step 7 and reading the meter for blanks, standards, and sample.

APPENDIX C

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