In the control of a sewage-disposal plant, the determination of the putrescibility is more important than the determination of the oxygen, and in comparison with the other constituents, the knowledge of the putrescibility or stability is of the greatest importance. With this test a plant can be controlled to better advantage than by any elaborate chemical analysis. The reason for this is simple. The ultimate aim of any disposal plant is to make the putrescible matter more or less stable. The putrescibility test indicates the degree of oxidation obtained, and the determination of the dissolved oxygen gives a fixed figure of the absolute amount available for further oxidation. Both factors are important in studying a nuisance or fish life. A high oxygen content, in itself, does not indicate stability.

Determination of Dissolved Oxygen.—In our laboratory, the Winkler method has been used to determine the dissolved oxygen in the manner described in the "Standard Methods of Water Analysis" adopted by the American Public Health Association. The sample is collected carefully in order to avoid aeration. Manganous sulphate and an alkaline solution of potassium iodine are then added. The precipitate of manganous hydrate is allowed to settle; sulphuric acid is then added, and the free iodine in the solution titrated with standardized sodium thiosulphate. The equivalent of free iodine is calculated to oxygen. The results are expressed either in p.p.m., or in percentage saturation. In the latter case the temperature must be considered. Normal fresh water which has not undergone temperature changes ought to be 100% saturated, but may be sub-saturated or super-saturated, depending upon conditions, such as the season of the year, the sunlight, the presence of algae, or sudden changes of temperature.

As an alternative method, the American Public Health Association has recently recommended the Levy method for the determination of dissolved oxygen. This consists, briefly, in the conversion of a ferrous salt into a ferric salt by the oxygen in the water. The residual ferrous salt is titrated with the permanganate solution. At present the use of either method is a matter of choice.

Determinations for dissolved oxygen should be made on the spot to avoid a loss of oxygen, particularly in putrescible samples. From a study of this question in our laboratory, we have concluded that only fresh saturated and uncontaminated water like that from Lake Michigan can be kept for a number of hours without showing a reduction in oxygen. Even then the sample should be kept in a cool, dark place. The addition of a small quantity of formaldehyde allows an extension of the storage period, but it is preferable to add the first two reagents in the Winkler method in order to form the precipitate, where the determination cannot be made on the spot.

Determination of Putrescibility.—The putrescibility test has been almost universally adopted by various sewage-purification plants. The one most in favor is the so-called methylene blue test devised by Spitta and Weldert in Germany. A dilute solution of methylene blue is added to a definite quantity of the effluent in a sterile bottle; the mixture is then incubated at 20° Cent. and the number of hours or days noted in which the blue color is discharged. This decolorization takes place only in putrescible samples and depends upon the formation of a leucobase when the oxygen in the sample has been exhausted. Some prefer an incubation temperature of 37° Cent. to obtain quicker results, but this temperature is not desirable because it does not conform to actual conditions as does the temperature of 20°. Furthermore a bacterial flora will flourish at 37°, which would not be favored at the lower temperature. Phelps has put the test on a quantitative working basis by which the putrescibility of a sample can be expressed in terms of relative stability. Such figures indicate the proportion of oxygen present as compared with the total amount required to effect the complete oxidation of a sample in question. The sample should be kept in the incubator for twenty days before being discarded, but from a practical working standpoint this is neither necessary nor desirable. Ten days storage as a maximum is sufficient, and often four days will give results sufficiently accurate for practical purposes. Samples which show signs of decolorization after four days may be held for further observation. The exact hour of decolorization is difficult to fix, but an experienced observer can avoid material mistakes in estimating the end point.

Index of Permissible Dilution.—The putrescibility test outlined is eminently practical and valuable, but is not a quick method. Recently Phelps devised another method to give the desired information in a shorter time. The test in principle is not new, for it has long been known that organic putrescible material will absorb oxygen in solution, and the rate of absorption under certain conditions has rightly been looked upon as a good index to the putrescibility of the sample. Only recently, however, could the permissible dilution be calculated for a sewage effluent under certain assumed conditions. The procedure is to mix a small quantity of sewage carefully with fresh aerated water in definite proportions, and to incubate the mixture at 20° Cent. for a number of hours. To hasten the result, the dilution may be lowered. The dissolved oxygen is determined in the mixture at the start and again after the lapse of several hours. With these two figures in hand, the percentage of sewage permissible in a stream under assumed conditions can be calculated. In the formula are terms for the period of contact and the percentage of dissolved oxygen to be available at the desired point in the flow. With our present knowledge of this test, the same assumptions must be followed from time to time in order to get comparable results, since the formula in its present state is not as perfect as may be, owing to the lack of knowledge of the shape of the curve for this bacterial reaction. The results obtained, however, are very practical and of great value. The method requires more accurate and careful work than the methylene blue test, and at present is open only to a skilled chemist.

Bacteria.—Most of the chemical changes in biologic sewage purification are in reality the result of bacterial activity. The significance of these bacteria in general is of interest largely from the scientific standpoint. The removal of disease-producing bacteria, however, is a question of practical interest. Sewage disposal devices, in themselves, are not built, primarily, as bacterial removers, and cannot be relied upon unless sterilization of the effluent with chloride of lime be added as a sure means for removing bacteria. In a chemical precipitation process, the bacteria may be reduced partly by sedimentation and partly by the germicidal properties of the chemicals employed. In a plain settling tank the reduction of bacteria is due to sedimentation. In a sprinkling filter or in a contact bed the reduction is undoubtedly the result of mechanical detention as well as the action of higher developed organisms. The unfavorable environment may also be an influence. Secondary settling basins will reduce the number of bacteria again by simple sedimentation. The effluent of a filter may contain as low as 5% of the initial number of bacteria present originally. Biologic sewage purification may eliminate the putrescible matter, but if bacterial purification be desired, disinfection only will secure the result.

In our laboratory the method employed to determine the number of bacteria is to place 1 c.c. of the sample, properly diluted, in a litmus lactose agar medium. The temperature of incubation is 37°, and the time of incubation two days for the routine samples. It is well known, however, that no artificial medium—be it an agar or a gelatine medium—will permit the growth of all bacteria ordinarily present. Therefore, the count per 1 c.c. indicates but a portion of the true number. More colonies will develop on gelatine than on agar.