Impact of Head Space on Test for Microbiological Growth (BS6920 Section 2.4)

Report to the Department of the Environment
IMPACT OF HEAD SPACE ON TEST FOR MICROBIOLOGICAL GROWTH
[BS6920 Section 2.4]
Report to the Department of the Environment

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SUMMARY

A recent study carried out for the American Water Works Association recommended some changes to Section 2.4 of BS6920 to improve the accuracy of the test. Briefly these changes were to increase the head space used in the test vessel, and to standardise the inoculum used in the test. To assess the relevance of one of these changes to the testing of materials in the UK the Department of the Environment, as part of Research Contract PECD 7/7/370 managed by the Drinking Water Inspectorate, asked WRc to investigate the influence of head space on the results obtained from the test method.

In the WRc study the impact of a range of head spaces, which included the current BS6920 value of 15% and the AWWA suggestion of 35%, were investigated. Several materials, which would normally be expected to give pass, fail and borderline responses, were used in the study. The effect of changes to the inoculum will be studied in another project.

The results of the study did not agree with the recommendations of the AWWA study, and produced the following conclusions:

1. Increase in head space reduces the sensitivity of the MDOD test.
2. No evidence that reproducibility is reduced at 15% head space.
3. Different acceptance criteria required for different head spaces.
4. Not all materials produce a plateau of DOD, therefore concern over a fixed period for MDOD determination.
5. Water change period affects MDOD result.

This study has demonstrated that the MDOD test in BS6920 Section 2.4 would not be improved by adopting the recommendations of the AWWA study.

The WRc study, whilst not confirming the results of the AWWA study, has suggested several ways in which the current MDOD method may be modified to improve its sensitivity. Methods being developed in Holland and Germany already have greater sensitivity, and if a more stringent acceptance criteria was to be agreed for the European Standard, then the current BS6920 Section 2.4 MDOD test would no longer be appropriate. Research is therefore required to improve the sensitivity of BS6920 so that it remains the standard method.

This study has suggested three ways in which sensitivity could be improved, which are as follows:

1. Reduction of the head space
2. Increase in the time between water changes
3. Increase in the ratio of surface area of material to volume of test water

It is recommended that these three options are investigated so that the UK is in a stronger position to promote the MDOD test procedure.
PREFACE

The leaching of organic substances from materials in contact with drinking water, which promote the growth of micro-organisms, can cause a deterioration in water quality. To ensure that only suitable materials are used in the construction of water distribution systems and fittings they must comply with BS6920. The influence which materials could have on microbial growth is addressed specifically by Section 2.4 of this standard.

A recent study carried out for the American Water Works Association recommended some changes to Section 2.4 of BS6920 to improve the accuracy of the test. Briefly these changes were to increase the head space used in the test vessel, and to standardise the inoculum used in the test. To assess the relevance of one of these changes to the testing of materials in the UK the Department of the Environment, as part of research Contract PECD 7/7/370 managed by the Drinking Water Inspectorate, asked WRc to investigate the influence of head space on the results obtained from the test method.

This report is an account of the work undertaken.
1. BACKGROUND

To remove trade barriers between member states, the European Commission has embarked on a programme to produce European standards which will replace the standards currently applied by individual member states. One of these standards will address the effects of materials on drinking water quality. There are several ways in which a material can be judged to be unsuitable for use because it influences water quality. One of these is to determine if materials can promote the growth of micro-organisms either on the surface of the material or in the main body of the water.

In the UK non-metallic products intended for use in contact with drinking water must comply with BS6920. Testing is required under the Water Byelaws Scheme on products used after the time of supply. The committee which advises the Secretary of State on approval matters requests the results of testing to BS6920 when considering an application for approval for use of a product in contact with public water supplies. This standard is split into several parts, each addressing a different parameter such as taste, appearance, toxicity and microbial effects. The influence due to microbial growth is dealt with in Section 2.4 - Growth of aquatic micro-organisms. In this test method the growth of micro-organisms resulting from a test material is determined by the magnitude of their consumption of dissolved oxygen (DO). The extent of growth is measured by determining the Mean Dissolved Oxygen Difference (MDOD) which essentially is the difference between the DO in the test container and that in the water blank. A further part of this test is the assessment of the potential for a material to support the growth of bacteria of public health concern.

UK manufacturers, as voluntary members of the Water Byelaws Scheme, produce products that meet the current BS6920 standard. It would therefore be desirable if the eventual European standard was based on BS6920 with the same acceptance criteria. DoE has therefore supported activities which might lead to the recognition of the testing procedures with CEN (the European Committee for Standardisation). To this end work within CEN has reached the position where it has been agreed that the MDOD part of BS6920 Section 2.4 should to go forward as a Pr-ENV. This will enable more countries to gain experience with it before hopefully it will form the basis of a European standard. Prenormative research has been deemed necessary to address problems associated with the measurements of the growth of bacteria of public health concern, and the potential improvement of the sensitivity of the test method.

In a recent American Water Works Association Research Foundation (AWWARF) report (Bellen et al. 1993) the researchers compared a version of the UK MDOD test with a method developed by Ellgas and Lee (1980). They found both methods to have some inconsistencies, but found the Ellgas and Lee method to be the most inconsistent. They also evaluated some modifications to the current BS6920 method which they suggested improved the consistency of the method. They concluded that the consistency of the method was improved by increasing the head space to 35%, and that more control of the inoculum concentration of the public heath indicator micro-organisms improved this aspect of the test. The American study has implications for the acceptance of the currently specified head space in the proposed European standard. The Department of the
Environment therefore required an independent study to confirm the American results and have funded this study as part of their contract with WRc on improved materials testing procedures (PEC D 77/370).

This report presents the details of the WRc trial to determine the effect of head space on the results obtained from the MDOD part of the test.
2. OBJECTIVES AND PROGRAMME OF WORK

As this study is only a part of contract PECD 7/7/370 it does not address all of the objectives of the contract and only adopts the relevant parts of the work programme defined in Schedule 1.

The objectives addressed are:

(a) To ensure the development of the best possible EC approval schemes.

(c) To establish testing needs, devise, validate and recommend modified testing procedures.

The relevant sections of the programme of work as defined in Schedule 1 of the contract are.

(a) The contractor will carry out, in agreement with the Department, validation of materials testing methods, including CEN collaborative tests, finalising test protocols and preparing appropriate forms/documentation.

(d) Provide general technical support to the Department on the development of acceptable EC standards for testing and approval of substances and products.
3. METHODS

3.1 Selection of test materials

The purpose of this trial was to determine the effect of a range of head spaces on the MDOD results obtained from test materials. As part of the initiative to produce a European standard for materials testing an interlaboratory trial has taken place in which materials with different abilities to promote growth were tested. The material chosen for this trial was an epoxy resin used in the rehabilitation of water mains. This material would not normally promote microbial growth, therefore to achieve a range of growth promoting ability the epoxy resin was formulated to contain different concentrations of benzyl alcohol. Preliminary trials had been carried out at Thames Water to ensure that the actual concentrations of benzyl alcohol chosen would produce three materials with the following desired growth promoting abilities:

1. A material that would pass the current MDOD test
2. A material that would be a borderline in the current MDOD test
3. A material that would fail the current MDOD test

The actual concentrations of benzyl alcohol that were selected were 2.4, 4.7 and 9.0%. The selected formulations were supplied in two parts, the resin and the hardener. For use in the trial these were mixed together in the appropriate volumes and coated onto stainless steel coupons.

As the selected materials were the same as those used in the interlaboratory trial, the results obtained at the standard head space in this study could be compared to those obtained by other laboratories using the MDOD test method.

For means of identification these three test materials were given the following code names:

- Epoxy resin containing 2.4% benzyl alcohol Mod 1
- Epoxy resin containing 4.7% benzyl alcohol Mod 2
- Epoxy resin containing 9.0% benzyl alcohol Mod 3

These code names are used in this report.

3.1.1 Application and curing of epoxy resin test materials

A standard procedure for coating the coupons with the test epoxy resin was adopted. This involved mixing the resin and hardener in the appropriate ratio. This mixture was then stirred thoroughly for two minutes to ensure complete mixing. The mixture was then painted onto the stainless steel coupons to a thickness of approximately 1 mm. The
prepared test plates were then allowed to cure for 16 hours at 20 °C. After the curing period the test plates were placed in running potable quality water for 1 hour. The coated plates were then ready for testing.

3.1.2 Additional materials

During a previous interlaboratory trial the UK laboratories obtained differing results for some of the materials even though they were all using the method described in BS6920. Tests were therefore carried out in this study on three of these materials at the standard 15% head space. The materials tested were a rubber, a plasticised PVC and a polyethylene.

3.1.3 Replication of testing

Duplicate flasks were set up for all materials and controls except for those carried out with the standard head space of 15% where triplicates were set up. An additional three flasks (with 15% head space) were set up for the wax positive control to give more information on replication at a relatively high MDOD, where the AWWA study had suggested poor reproducibility.

3.2 Selection of test head space

The current standard MDOD test is carried out in 1 litre (nominal capacity) preserving jars. Test material with a surface area of 15 000 mm$^2$ is placed in the preserving jar and the jar is filled to the 1 litre volume mark with test water. When the jar is sealed with its lid there is an effective head space of 15%. The American Water Works Association (AWWA) study recommended a head space of 35%. To give a range of head spaces that covered both the UK and AWWA recommendations, the following head spaces were chosen for this trial: 15, 25, 35 and 45%.

It was considered important that in the AWWA study the surface area to volume ratio of the test material to test water should be maintained constant at the different head spaces. To achieve this it was necessary to coat stainless steel coupons at the four desired surface areas with the epoxy resin test materials.

3.3 Test control materials

Control flasks were set up of the test water, paraffin wax (positive control) and as a negative control the stainless steel used as a support for the epoxy resin. These controls were set up at each of the selected head spaces to give the same surface area to volume ratios applied to the test materials. To ensure that the positive control had the correct surface area, stainless steel coupons with the chosen surface areas were dipped in molten paraffin wax control material to produce a coating of approximately 1 mm thickness.
3.4 Test Water

The test water used in this study was a high quality borehole water obtained from a supply at the WRC Medmenham laboratory. Chemical analysis of this water revealed that it required supplementing with nitrogen and phosphate to meet the essential nutrient concentrations specified in BS6920 Section 2.4. Therefore at each water change the test water was supplemented with appropriate concentrations of Analar grade potassium nitrate and potassium dihydrogen phosphate to bring their effective concentrations up to the minimum levels specified in BS6920 Section 2.4.

3.5 Calibration of dissolved oxygen determination

Dissolved oxygen (DO) measurements were carried out using a YSI 58 DO Meter and a self stirring BOD bottle DO probe. The DO meter and probe were calibrated before each days DO measurements using the Winkler titration procedure as described in Methods for the Examination of Waters and Associated Materials, ‘Dissolved Oxygen in Natural and Waste Waters 1979’ (SCA 1980).

3.6 Experimental protocol

On the start day of the test procedure the test and control materials were placed in separate test containers. The appropriate volumes of test water and inoculum water were then carefully decanted into each flask in the volumes specified in Table 3.1.

Table 3.1 Surface areas of materials and volumes of test water and inoculum used to set up test procedure

<table>
<thead>
<tr>
<th>Head space (%)</th>
<th>Surface area of material (mm²)</th>
<th>Volume of test water (ml)</th>
<th>Volume of inoculum (ml)</th>
<th>Surface area to volume ratio (mm²/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>15370</td>
<td>900</td>
<td>100</td>
<td>15.4</td>
</tr>
<tr>
<td>25</td>
<td>13540</td>
<td>800</td>
<td>90</td>
<td>15.2</td>
</tr>
<tr>
<td>35</td>
<td>11710</td>
<td>690</td>
<td>80</td>
<td>15.2</td>
</tr>
<tr>
<td>45</td>
<td>9880</td>
<td>585</td>
<td>65</td>
<td>15.2</td>
</tr>
</tbody>
</table>

The test flasks including those containing control materials and the test water control at each of the specified head spaces were placed in a 30 °C incubator.

The test protocol specified in BS6920 Section 2.4 requires that twice weekly the water in each flask should be carefully decanted, and replaced with an equal volume of fresh test water the same as applied at the start of the test procedure. The flask should then be resealed and the incubation at 30 °C continued. The water changes were made at 3 and 4 day intervals for the complete 7 weeks of the test.
In the full test procedure described in BS6920 Section 2.4 dissolved oxygen determinations are carried out only at the first water change of each of the last 3 weeks of the test. The microbiological tests are carried out at the other water change during the last 3 weeks of the test. In this study as no tests were required for the growth of coliforms or *Pseudomonas aeruginosa* additional dissolved oxygen determinations were carried out at the second water change during the last 3 weeks of the test. Additionally to give more information on the changes that occur in the MDOD profile through the whole test period, dissolved oxygen determinations were carried out at every water change after the first week up to the fifth week. Dissolved oxygen determinations were carried out as soon as each flask was opened prior to the water change.

### 3.7 Calculation of MDOD

Calculation of the MDOD of a test material was carried out as follows. The difference in dissolved oxygen (DOD) between the test material and that obtained from the test water control flask is calculated for each of the DO measurements made at the first water change in the last 3 weeks of the test. These three results are then averaged to give the MDOD.

A test sample that produces an MDOD value of less than 2.4 mg l\(^{-1}\) is considered not to support appreciable microbial growth with the following qualification. When a single sample produces an MDOD value in the range 1.7 mg l\(^{-1}\) to 2.9 mg l\(^{-1}\), then two further samples of the product should be examined. If the mean of the three determinations produces and MDOD value which is less than 2.4 mg l\(^{-1}\), then the material is considered acceptable.

In this study DO measurements were made at all water changes after the first week to give more information of the profile of DO consumption. Each days result is expressed as a simple difference in DO between the test sample and the test water control.
4. RESULTS AND OBSERVATIONS

4.1 Effects of head space on MDOD and DO results

The MDOD results obtained from the test epoxy resin materials supplemented with a range of concentrations of benzyl alcohol are presented in Table 4.1. From these results it can be seen that an increase in head space produces a lowering of the MDOD. This effect is most pronounced in the results obtained from sample Mod 3 which contained the highest concentration of benzyl alcohol.

Table 4.1 MDOD results from test epoxy resin samples

<table>
<thead>
<tr>
<th>Head space</th>
<th>Mod 1 Mean</th>
<th>Mod 1 Std dev</th>
<th>Mod 2 Mean</th>
<th>Mod 2 Std dev</th>
<th>Mod 3 Mean</th>
<th>Mod 3 Std dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.45</td>
<td>0.11</td>
<td>3.34</td>
<td>0.40</td>
<td>5.15</td>
<td>0.37</td>
</tr>
<tr>
<td>25</td>
<td>0.25</td>
<td>0.04</td>
<td>1.95</td>
<td>0.28</td>
<td>3.63</td>
<td>0.38</td>
</tr>
<tr>
<td>35</td>
<td>0.41</td>
<td>0.14</td>
<td>2.05</td>
<td>0.22</td>
<td>3.88</td>
<td>0.48</td>
</tr>
<tr>
<td>45</td>
<td>-0.03</td>
<td>0.03</td>
<td>1.99</td>
<td>0.19</td>
<td>2.54</td>
<td>0.47</td>
</tr>
</tbody>
</table>

A similar effect to that obtained from the test materials can be seen in the results obtained from the positive control material (Table 4.2). There was no obvious effect of head space on the stainless steel negative control, however, these values were very low and at the limits of sensitivity of DO measurement.

Table 4.2 MDOD results from control samples

<table>
<thead>
<tr>
<th>Head space</th>
<th>Paraffin wax Mean</th>
<th>Paraffin wax Std dev</th>
<th>Stainless steel Mean</th>
<th>Stainless steel Std dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>6.50</td>
<td>0.32</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>25</td>
<td>5.79</td>
<td>0.33</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>35</td>
<td>4.66</td>
<td>0.52</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>45</td>
<td>4.52</td>
<td>0.06</td>
<td>-0.22</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Results of the DO measurements carried out on the negative control flasks at the different head spaces are presented in Table 4.3. The results for the test water and stainless steel appear to show a small decrease in DO as the head space is increased. This is slightly surprising, as it might be expected that the effect of oxygen in the head space would be to increase the DO concentration in the water.

Table 4.3  Average dissolved oxygen results from negative control samples

<table>
<thead>
<tr>
<th>Head space</th>
<th>Test water</th>
<th>Stainless steel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std dev</td>
</tr>
<tr>
<td>15</td>
<td>7.95</td>
<td>0.25</td>
</tr>
<tr>
<td>25</td>
<td>7.72</td>
<td>0.25</td>
</tr>
<tr>
<td>35</td>
<td>7.64</td>
<td>0.25</td>
</tr>
<tr>
<td>45</td>
<td>7.37</td>
<td>0.25</td>
</tr>
</tbody>
</table>

4.2  Comparison of MDOD results with those of other UK test laboratories

The results obtained by the three UK test laboratories involved in the interlaboratory trial of the epoxy resin materials and those obtained at WRc during this study are given in Table 4.4. These results were obtained at the standard 15% head space.

Table 4.4  Results of MDOD from UK laboratories

<table>
<thead>
<tr>
<th>MOD 1</th>
<th>MOD 2</th>
<th>MOD 3</th>
<th>+ve control</th>
<th>-ve control</th>
<th>-ve reference (MDO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRc</td>
<td>Lab A</td>
<td>Lab B</td>
<td>WRc</td>
<td>Lab A</td>
<td>WRc</td>
</tr>
<tr>
<td>0.5</td>
<td>1.2</td>
<td>0.5</td>
<td>0.5</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>3.3</td>
<td>3.1</td>
<td>1.0</td>
<td>3.3</td>
<td>3.1</td>
<td>1.0</td>
</tr>
<tr>
<td>5.2</td>
<td>4.6</td>
<td>4.3</td>
<td>5.2</td>
<td>4.6</td>
<td>4.3</td>
</tr>
<tr>
<td>5.6</td>
<td>7.0</td>
<td>6.8</td>
<td>6.5</td>
<td>7.0</td>
<td>5.3</td>
</tr>
<tr>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>7.9</td>
<td>7.5</td>
<td>7.6</td>
<td>7.9</td>
<td>7.5</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.3</td>
</tr>
</tbody>
</table>
These results indicate that there was some agreement between the results obtained. However, for Mod 2, two laboratories would fail the material whilst the others would pass it, and Lab C would only border-line fail Mod 3. It must be noted however that the +ve control results of 5.3 mg l\(^{-1}\) obtained from Lab C was outside the accepted range of 5.5 to 8.5 mg l\(^{-1}\) specified in BS6920, and therefore would invalidate the test results obtained.

The results obtained from the materials that had been used in a previous CEN interlaboratory trial are given in Table 4.5. These show good agreement between the WRc results and the other laboratories for the rubber and polyethylene materials. However, the WRc result for the plasticised PVC material only agrees closely with that obtained from one of the UK test laboratories, but agrees with the French laboratory in Lille that the material would fail BS6920. There is considerable variability in the results obtained from the various laboratories for this particular material.

<table>
<thead>
<tr>
<th>Table 4.5</th>
<th>Comparison of MDOD results obtained from previously tested materials in CEN interlaboratory trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WRc</td>
</tr>
<tr>
<td>Rubber sheet</td>
<td>1.7</td>
</tr>
<tr>
<td>Orange pPVC sheet</td>
<td>5.6</td>
</tr>
<tr>
<td>Black PE sheet</td>
<td>0.2</td>
</tr>
</tbody>
</table>

### 4.3 Dissolved oxygen difference profiles over seven week period

The effects of head space on the results obtained from the twice weekly determinations of dissolved oxygen difference (DOD) on the epoxy resin materials over the seven weeks of the test are presented in Figures 4.1, 4.2 and 4.3. Apart from material Mod 1 there is an obvious difference between the DOD profiles obtained at a 15% head space and the others. There is also a similar trend to that obtained from the actual MDOD results (Table 4.1) of a decrease in DOD with increase in head space. Of more significance is the shape of the profile with all three materials demonstrating an initial high DOD which gradually decays over the seven week period of the test.

These profiles are very different to those obtained from the control materials, which are presented in Figures 4.4 and 4.5. Although there is a distinct difference between the DOD values obtained at the four head spaces for the paraffin wax positive control, this material produces an almost plateau response from the first week. A similar response is obtained from the stainless steel negative control, however, attention must be drawn to the difference in the scale of DOD results. All the points on the graph are close to the limits of detection of this procedure, and are not as variable as would appear from a simple visual comparison with the other figures, because of the expanded axis used in this graph.
Figure 4.1 Effect of head space on DOD - sample MOD 1

Figure 4.2 Effect of head space on DOD - sample MOD 2
Figure 4.3 Effect of head space on DOD - sample MOD 3
Figure 4.4 Effect of head space on DOD - paraffin wax positive control

Figure 4.5 Effect of head space on DOD - stainless steel negative control
To further emphasis the different DOD profiles that materials exhibit, the results of the DOD determinations on the rubber, plasticised PVC and polyethylene materials are presented in Figure 4.6. Each of these materials has a different profile, the polyethylene has a very low response and produces a plateau close to the limit of detection from the first day of monitoring. The plasticised PVC also produces a good plateau, but requires three weeks before the plateau is reached. The rubber material on the other hand does not reach a plateau, but exhibits an almost linear decay in DOD.

![Graph of DOD vs. Days for Rubber, Polyethylene, and pPVC](image)

**Figure 4.6  DOD of samples from previous trial**

### 4.4 Effect of head space on replication

One of the main reasons for the AWWA studies recommendation for increasing the head space was that they found reproducibility was improved. The results of replicate determinations at the four head spaces are presented in Figures 4.7, 4.8, 4.9 and 4.10.

Although not conclusive it would appear that at a 15% head space there was more variability, especially with the material with the highest growth response (Mod 3), but most of this variability appears to take place before the last three weeks of the test, the period when the MDOD is determined.

To determine whether either the head space or test material affected the reproducibility of the MDOD results, the inverse of the coefficients of variation of the results obtained from each test material at each of the head spaces were subjected to a two way analysis of variance. The analysis confirms that there was no significant difference between reproducibility associated with either head space (p value 0.58) or test material (p value 0.13) at the 5% confidence level.

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Figure 4.7  DOD replication at 15% head space

Figure 4.8  DOD replication at 25% head space
Figure 4.9 DOD replication at 35% head space

Figure 4.10 DOD replication at 45% head space
4.5 Effect of water change period on MDOD

A feature of this study was the effect that the different periods of time between water changes each week had on the DOD results. There is a very noticeable saw-edge effect to the DOD profiles, particularly with the 15% head space. This effect is seen most obviously in Figures 4.2 and 4.3 for materials MOD 2 and MOD 3, but is a feature in almost all of the DOD profiles. This is the effect of the different contact times of the test water with the material. The longer contact period of 4 days giving a higher DOD than that observed after the 3 day water change. The MDOD is the average of either the 3 day or 4 day DOD results during the last 3 weeks of the test. The resulting MDOD will have a higher value when based on the 4 day water change DOD values, than that given from 3 day data.

There is obviously a need to standardise the method for DO measurement on one or other of the 3 or 4 day water changes. Currently two test laboratories use 3 days and the other 4. The WRc figures quoted in this report are 3 day results.
5. DISCUSSION AND ANALYSIS

In the AWWARF study it was found that if the head space was too large or too small then oxygen transfer and uptake rates had a significant effect on the reproducibility of the method. Best results were obtained at a head space of 35%, where it is suggested that the combination of oxygen transfer and uptake rates were optimal. The study recommends that the BS6920 MDOD protocol and acceptance criteria should be used with the exception that the head space should be increased to 35%. However, the results of the study reported here did not support these findings.

In the WRc study there was some evidence of more variability at the 15% head space during the first 4 weeks of the test period, but this was not apparent during the important final 3 week period when the data for MDOD assessment is obtained. Statistical analysis confirmed that there was no significant difference in reproducibility associated with head space (p value 0.58) at the 5% confidence level.

Of more significance was the reduction in sensitivity that occurred when the head space was increased (Table 4.1). Without exception the highest MDOD values for a material were achieved at the 15% head space. The apparent improvement in reproducibility suggested in the AWWA study may have been attributable to the fact that sensitivity had been reduced which may have given an apparent improvement in variability.

The reduction in sensitivity has an even more serious impact if the same acceptance criteria is maintained independent of head space as suggested in the AWWA study. This would almost certainly allow materials to be deemed acceptable at a 35% head space that would fail the current BS6920 Section 2.4. This effect is demonstrated well with test material MOD 2 (Table 4.1) where at the BS6920 head space (15%) the MDOD was 3.34 mg l\(^{-1}\), whereas at all the other head spaces the material was a borderline pass with MDOD values around 2 mg l\(^{-1}\), which is below the 2.4 mg l\(^{-1}\) limit.

Another feature of this study has been the interesting results obtained from following the DOD profile over the whole test period. Normally 3 readings are taken over the last 3 weeks of the test and these are used to calculate the MDOD. The first feature of these profiles was that the different materials produced very different profiles. Both the positive and negative control materials produced plateau responses (Figures 4.4 and 4.5) almost from the first reading in week 1. On the other hand almost all of the test materials in this study, with the exception of polyethylene, did not produce an immediate plateau. Many of the materials had a profile which was producing a reducing DOD over the last 3 weeks of the test period. There was also evidence that a material may have very poor DOD values for the first 3 weeks of the test, but may produce an acceptable MDOD by the end of the test. There may therefore be a need for more flexibility in the application of the MDOD criteria.

The other feature demonstrated by the profiles was the effect that the period between water changes had on the DOD results. There is clear evidence that the DOD value obtained at the 4 day water change was higher than that achieved after 3 days. This is not surprising since it would be expected that the amount of dissolved oxygen consumed would be related to contact time of the micro-organisms with the test material and test water. It is therefore very important that the actual contact period should be defined in the MDOD test.
This study has demonstrated that the current BS6920 Section 2.4 MDOD test would not be improved by an increase in head space, but would in fact be made less sensitive. A criticism which has been made of the MDOD test is that it lacks sensitivity. This trial has demonstrated two ways in which sensitivity may be improved. Firstly, there is an obvious relationship between head space and sensitivity, it may be possible to improve sensitivity by further reducing the head space to less than 15%. Secondly, the effect of water change period (3 or 4 day) has been demonstrated to effect sensitivity, higher MDOD values were obtained from 4 day rather than 3 day water changes. An increase to 7 days, or maybe longer, would be predicted to improve sensitivity. Another alternative to improve sensitivity would be to increase the surface area of material in contact with the test water.

Any improvement in sensitivity would of necessity require an increase in the acceptance criteria value of 2.4 mg l\(^{-1}\), to take account of the larger MDOD values that would be obtained. Conversely, the AWWA recommendations reduce sensitivity and therefore would require a lower MDOD value as an acceptance criteria to maintain parity with BS6920 Section 2.4.

This study has demonstrated that the MDOD test in BS6920 Section 2.4 would not be improved by adopting the recommendations of the AWWA study to increase the head space. It has, however, suggested several ways in which the sensitivity of the test could be improved, these options are worthy of further research.
6. CONCLUSIONS

1. Increase in head space reduces the sensitivity of the MDOD test.

2. No evidence that reproducibility is reduced at 15% head space.

3. Different acceptance criteria required for different head spaces.

4. Not all materials produce a plateau of DOD, therefore concern over a fixed period for MDOD determination.

5. Water change period affects MDOD result.
7. RECOMMENDATIONS

The WRc study, whilst not confirming the results of the AWWA study, has suggested several ways in which the current MDOD method may be modified to improve its sensitivity. Methods being developed in Holland and Germany already have greater sensitivity, and if a more stringent acceptance criteria was to be agreed for the European Standard, then the current BS6920 Section 2.4 MDOD test would no longer be appropriate. Research is therefore required to improve the sensitivity of BS6920 so that it remains the standard method.

This study has suggested three ways in which sensitivity could be improved, which are as follows:

1. Reduction of the head space
2. Increase in the time between water changes
3. Increase in the ratio of surface area of material to volume of test water

It is recommended that these three options are investigated so that the UK is in a stronger position to promote the MDOD test procedure.
REFERENCES


British Standards Institution (1988) Suitability of materials for use in contact with water for human consumption with regard to their effect on the quality of the water. BS6920 Section 2.4, London.