

THE OXYGEN UPTAKE BY PSEUDOMONAS FLUORESCENS ON GLUCOSE,
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XYLOSE, ARABINOSE AND ACETATE UNDER VARYING CONDITIONS OF
SUBSTRATE CONCENTRATION AND ENVIRONMENTAL TEMPERATURE

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TABLE OF CONTENTS

	Page
I. INTRODUCTION	7
II. LITERATURE REVIEW	10
History of Waste Disposal	10
Problem of Industrial Wastes	11
Biological Aspects	23
Bacterial Respiration	29
Oxygen Requirements of Bacteria	41
III. EXPERIMENTAL	63
Purpose of Investigation	63
Plan of Experimentation	64
Materials	65
Apparatus	68
Method of Procedure	72
Data and Results	84
Sample Calculations	99
IV. DISCUSSION OF RESULTS	101
Discussion	101
Recommendations	132
Limitations	141
V. CONCLUSIONS	146

	Page
VI. SUMMARY	154
VII. BIBLIOGRAPHY	159
Addenda	171
VIII. ACKNOWLEDGMENTS	172
IX. VITA	173

FIGURES

	Page
Figure 1. Bacterial Growth Curve	30
Figure 2. Warburg Constant Volume Manometer and Flask	46
Figure 3. Calibration Curve for Klett-Summerson Colorimeter for Conversion of Colorimeter Scale Readings to Dry Weight of <u>Pseudomonas Fluorescens</u> per Milliliter of Aqueous Suspension	87
Figure 4. Effect of Concentration of Substrate on the Rate of Oxygen Uptake by <u>Pseudomonas Fluorescens</u>	91
Figure 5. Effect of Temperature on the Rate of Oxygen Uptake by <u>Pseudomonas</u> <u>Fluorescens</u>	93
Figure 6. Effect of Concentration of Substrate on the Rate of Oxygen Uptake by <u>Bacillus Subtilis</u>	96
Figure 7. Effect of Temperature on the Rate of Oxygen Uptake by <u>Bacillus Subtilis</u>	98

TABLES

		Page
Table I	Types of Industrial Wastes	13
Table II	The $Q_{(O_2)N}$ of Genus <u>Micrococcus</u> on Various Substrates	59
Table III	Colorimeter Readings and Weights of Dry Bacterial Protoplasm for Distilled Water Suspensions of <u>Pseudomonas Fluorescens</u>	86
Table IV	Weights of Mercury Contained by Warburg Flasks and the Calculated Volume of the Flasks	88
Table V	Weights of Mercury Contained by Manometers During Calibration, and the Calculated Manometer Volumes	89
Table VI	Effect of Concentration of Substrate on the Rate of Oxygen Uptake of 3.0 Milligrams of <u>Pseudomonas Fluorescens</u> Suspended in 2.5 Milliliters of 0.05 Molar Phosphate Buffer of pH 6.8 at a Reaction Temperature of 25 °C	90
Table VII	Effect of Temperature on the Rate of Oxygen Uptake of 3.0 Milligrams of <u>Pseudomonas Fluorescens</u> Suspended in 2.5 Milliliters of 0.05 Molar Phosphate Buffer of pH 6.8	92
Table VIII	Total Oxygen Uptake of 2.8 Milligrams of <u>Bacillus Subtilis</u> Respiring on Varying Amounts of Glucose and Sodium Acetate in a 2.5 Milliliter Volume at 25 °C	95

	Page
Table IX The Oxygen Uptake of 2.8 Milligrams of <u>Bacillus Subtilis</u> on Glucose and Sodium Acetate Substrates at 20, 21, 22.5, 25 and 30 °C	97

I. INTRODUCTION

The rivers and streams of the land have long been the willing servants of man and his civilization. They have served as arteries of commerce, suppliers of water and power, and as collection and disposal agencies for wastes. However, when rivers are misused for waste disposal, they become polluted, useless, a hazard to life and property, and offensive to both sight and smell. Since selfishness and ignorance have all too frequently led to this state of pollution, many governments are passing and enforcing strict laws and rules concerning the use of waterways for the disposal of wastes.

Industrial operations give rise to many types of wastes. Frequently these wastes are disposed of by simply adding them to some river or lake. Indeed, a river used intelligently to remove industrial wastes is as much of a natural resource and asset as it is when used as a means of transportation for the useful goods of commerce. However, if the wastes of an industry are considered a nuisance and cause damage to the stream, either real or esthetic, the industry is almost certain to have legal action taken against it. It is therefore the problem of such industries to take the necessary steps to alleviate pollution.

An industry faced with a pollution problem has three general solutions it may attempt. If the waste contains useful by-products, the industry may attempt to recover these materials and thereby cut down on its waste load. The industry may attempt a redesign of its processing with the idea of cutting wastes to a minimum. Last, the industry may attempt to devise some means of treatment for the wastes to make them nonoffensive before discharging them.

Inorganic waste problems are not usually difficult as these materials can frequently be rendered neutral, nontoxic, soluble and colorless by simple chemical treatment. Organic wastes pose an additional problem in that they are oxidized in the stream by the normal microbiological population or by microorganisms carried by the waste, bringing about a depletion of dissolved oxygen. Unfortunately, very little fundamental information is available on the effect of concentration, temperature, and catalysis, in the form of one waste acting upon another, under the biologically oxidative conditions existing in the stream. Considerable fundamental work must be done in this field before any intelligent evaluations of the effect of organic wastes can be made.

The pulp and paper industry has a typical organic pollution problem. Wastes from this industry have a high oxygen demand which is caused principally by acetate and pentosans. Pollution

caused by these wastes increases rapidly with increasing temperature of receiving waters. This problem is quite critical and more worthy of fundamental investigation.

The purpose of this investigation was to obtain fundamental information concerning stream pollution by studying the effect of substrate concentration and temperature of environment on the rate of oxygen utilization by a pure culture of a typical stream bacterium, Pseudomonas fluorescens. The substrates studied were glucose, xylose, arabinose, and acetate, chemicals of known structure and representative materials of wood pulp waste.

II. LITERATURE REVIEW

A search of the literature was made to determine previous work in the fields of stream pollution, waste disposal, and fundamental methods of determining biological oxygen uptake. Journals and manuscripts in the fields of chemical engineering, sanitary engineering, chemistry, biochemistry, and bacteriology were surveyed. The literature will be discussed under the following general headings: history of waste disposal, the industrial problem, the biological aspects of the problem, bacterial respiration, and oxygen requirements of bacteria.

History of Waste Disposal

The history of waste disposal begins with the disposal of storm waters and street rubbish in the sewers of the great cities of antiquity. The ancient city of Rome is known to have had an excellent sewer system which was well managed. After the fall of the Roman Empire the practices of waste disposal degenerated, and no further progress was made until the middle of the nineteenth century. At that time, public indignation at the stench and disease so prevalent in larger cities, brought about a revival in sewer construction and waste disposal^(1,49). As soon as effective sewers had been constructed, and the wastes of the cities were being discharged into nearby waterways, the second

problem of stream pollution appeared. This problem was immediately attacked, and led to the development of such sewage treatments as the trickling filter, the septic tank, the activated sludge process^(1,49), and others.

Industrial waste disposal and waste treatment is a problem of the twentieth century. Industrial expansion, and the effluents from these industries has added considerably to the stream pollution problem. Little attention was paid to industrial wastes prior to 1920⁽²⁴⁾, though the Royal Commission on Sewage⁽³³⁾ in England did some experimental work on the disposal of straw pulp waste by trickling filters in 1915. The first real interest in the disposal of industrial wastes occurred in the United States in the years of 1929 and 1930⁽¹⁸⁾.

Problem of Industrial Wastes

In the following section some of the more important aspects of the problem of industrial wastes and waste disposal will be discussed.

The Industrial Problem. Any industry has as its principal problem the production of some article of commerce at a profit. In order to do this, it is requisite that it, like any other dynamic entity, live in peace with its neighbors. Therefore,

it is necessary that industrial wastes be disposed of with the greatest possible economy and at the same time avoid causing any nuisance⁽³⁾.

Types of Industrial Wastes. Industrial wastes, as it is pointed out by Eldridge⁽²⁵⁾ and Phelps⁽⁶⁰⁾, are highly diversified in nature, and no over-all classification can be given, except in the most general terms. Besselièvre⁽⁵⁾ uses the simple classification of wastes as merely solid, liquid, and gas. Gaseous wastes are usually discharged into the atmosphere, and constitute the separate field of atmospheric pollution. Most solid and liquid wastes find their way into the streams and rivers of the land, and are the important wastes in the field of stream pollution. Phelps⁽⁶⁰⁾ classifies these wastes as organic and inorganic. Eldridge⁽²⁵⁾ classifies wastes as wastes from which valuable by-products can be extracted, and wastes of no value. Numerous other classifications and sub-classifications have been proposed, but for the most part these classifications depend on the particular interest of the classifier. One such classification due to Ettinger⁽²⁹⁾ is given in Table I. In general, it is sufficient to say that each industry has a waste peculiar unto itself, and each waste represents an individual problem^(3,25,60).

TABLE I

Types of Industrial Wastes

- A. Wastes Principally Mineral in Nature
 - 1. Brine wastes
 - 2. Mine wastes
 - 3. Waste slurries
 - 4. Mineral washing slurries and suspensions
 - 5. Pickle liquor wastes
 - 6. Plating wastes
 - 7. Miscellaneous wastes from inorganic chemical manufacture

- B. Wastes that Contain Principally Organic Matter
 - I. Hydrocarbon wastes
 - 1. Oil wells
 - 2. Petroleum refineries
 - 3. Styrene manufacturing plants
 - 4. Copolymer rubber plants
 - 5. Butadiene manufacturing plants
 - 6. Natural rubber processing or reclaiming
 - 7. Gasoline filling stations, bulk stations, garages, etc.
 - II. Miscellaneous organic chemical wastes
 - 8. Munitions plants such as TNT, tetryl, and ammonium picrate manufacture
 - 9. Pharmaceuticals (synthetic)
 - 10. Synthetic fiber plants such as viscose or nylon
 - 11. Organic chemical manufacture
 - III. Phenolic wastes
 - 12. Gas plants
 - 13. By-product coke plants
 - 14. Chemical plants
 - 15. Synthetic resin plants (phenolic resins)
 - 16. Tar, road oil and creosoting plants
 - 17. Wood distillation plants
 - 18. Dye manufacturing plants
 - IV. Biological Wastes
 - a. Wastes from processing biological materials and/or biological processes
 - 19. Tanneries
 - 20. Pharmaceuticals (Antibiotics, biologicals)
 - 21. Alcohol industries (breweries and distilleries)
 - 22. Miscellaneous fermentation industries
 - 23. Glue and gelatin plants
 - 24. Wool scouring
 - 25. Textile manufacture
 - 26. Paper manufacture, particularly sulfite pulp and kraft mills
 - 27. Laundry wastes
 - b. Food processing wastes
 - 28. Canneries
 - 29. Meat packing
 - 30. Milk and dairy products plants
 - 31. Corn products plants
 - 32. Beet sugar plants
 - 33. Cane sugar plants
 - 34. Fish processing plants
 - 35. Other food processing and dehydration plants

Ettinger, M. B.: Analytical Procedures for Industrial Wastes, Water and Sewage Works, 97, 292-294 (1950).

Legal Aspects. The legal aspects of industrial waste disposal are concerned with discharge of wastes into sewers and the pollution of streams⁽³⁾. Legislation concerning the discharge of wastes into sewers is municipal in origin and concerns itself principally with preventing corrosion of sewers and treatment plants. Municipalities sometimes enact legislation that prevents discharge of wastes into sewers when the wastes interfere with the correct operation of treatment plants.

Legislation concerning stream pollution is state or federal in origin^(19,53,54). Most states now have legislation designed to reduce pollution or prevent other new pollution. The laws vary considerably and are at present in a state of considerable flux⁽¹¹⁷⁾. Some attempts are being made with interstate agencies to give more uniformity in action. An example of this is the Ohio River Valley Water Sanitation Commission which is composed of members from the states of Illinois, Indiana, Kentucky, New York, Ohio, Pennsylvania, Virginia, and West Virginia⁽¹⁹⁾.

Stream pollution legislation is useless unless enforced and enforceable. Enforcement comes about best by cooperation between state and industry⁽³⁾. Some states realize this fact. A fine example is Washington, which publishes a very reasonable set of minimum standards for industrial effluents⁽⁵³⁾. Industry, too,

is vitally interested and has as one of its most important joint efforts the "Stream Pollution Abatement Committee" of the Manufacturing Chemists Association of the United States, Incorporated⁽³⁸⁾. This committee and its numerous sub-committees, which are voluntary and industrially supported, have as their aim the study and abatement of stream pollution.

An interesting study in the legal aspects of stream pollution is presented by Cleary⁽¹⁹⁾, chief commissioner for the Ohio River Valley Sanitary Commission. An industry which is designated as Industry "X", petitioned for permission to discharge the wastes of a proposed plant into the upstream area of the Ohio River. Upon study it proved that the wastes would cause considerable hardness in the waters. Since Industry "X" did not suggest treatment, it was assumed that none was intended, so the petition was refused. The industry promptly gave up the proposed plant, much to the disappointment of the community which had spent considerable effort in attempting to persuade the company to come into this area. Other industries, likewise, objected on the grounds that stringent pollution regulations put the whole valley at a competitive disadvantage. Cleary, however, pointed out that regulations are becoming stricter everywhere, and the disadvantage does not really exist. He then states that the industry might have been able to extract a valuable by-product from its waste.

Mention was given of one industrialist who stated that a sanitary commissioner, by forcing his company to clean up its waste, actually caused his concern to realize a profit from waste recovery. To further this argument the vitamin recovery and cattle feed manufacture realized by some of the fermentation industries was also brought out.

This story is, of course, somewhat one-sided, but worthy of some discussion. One is first deeply impressed by the apparent lack of cooperation between the industry and the commission. A meeting might easily have resolved the difficulties to the profit of all concerned. It certainly seems that such a meeting could have caused no harm, and was well worth a try. It could have easily been instigated by either side, particularly by the commission while it was studying the petition. Cleary's answer to the industrial objections of a competitive disadvantage was good in regard to the fact that anti-pollution laws are becoming universal. However, his statements concerning valuable by-products recovery show considerable lack of insight into the problems of the chemical industry. A few industries, such as those he cites, do have valuable by-products in their primary effluent, but more frequently the products present are so dilute and/or valueless that they will not begin to pay for their

recovery. If one could assume from the case of Industry "X" that the objectionable hardness would be caused by either calcium or magnesium salts, it is highly doubtful that any valuable by-products could be recovered.

Methods of Analysis. In order to comply with the laws of the land and to control their waste effluent, industries must make certain analyses of their wastes. The generally approved sanitary analyses consist of the following tests⁽²⁹⁾: biological oxygen demand, oxygen consumed (chemical oxygen demand), nitrogen determinations, settleable solids, total solids, volatile matter, pH, temperature, and dissolved oxygen. For industrial wastes other special tests are made, such as tests for color, hardness, and toxicity. In order to apply these tests successfully, Ettinger⁽²⁹⁾ points out that one must establish the purpose to be served by the information obtained, specify the tests to be used very carefully, and continually recheck the efficiency of the procedures.

The procedures used are standardized and for the most part are rather simple. Suspended solids are determined by filtration, total solids by evaporation, and dissolved solids by the difference between suspended and total solids⁽¹⁰⁴⁾. Volatile matter is determined by ignition at low red heat⁽¹⁰⁴⁾. The pH is determined colorimetrically or potentiometrically⁽¹⁰⁵⁾.

Temperature is, of course, determined with thermometer or thermocouple. Ammonia nitrogen is determined by the Kjeldahl method; nitrite and nitrate nitrogen are usually determined colorimetrically⁽¹⁰⁶⁾. Dissolved oxygen is normally determined by the Winkler method⁽¹⁰⁷⁾. The biochemical oxygen demand and oxygen consumed tests are a little more involved and specialized and will be discussed in the next paragraph.

Biochemical Oxygen Demand. The biochemical oxygen demand measures the oxygen used by microorganisms in oxidizing a sample of waste. The microorganisms are either present in the waste, as in the case of sewage, or are added to the waste, as is frequently the case in industrial wastes. In either case, it is hoped that the organisms present represent a population similar to the population that will oxidize the waste in the stream. The procedure used is as follows^(107,109): a measured sample of the waste is placed in a known quantity of dilution water. The dilution water is usually unchlorinated, buffered surface water which has been completely aerated. The mixed water and sample are then placed in a tightly stoppered bottle, with no air left above the liquid, and are incubated at 20 °C for a period of five days. A sample of the dilution water is also incubated under the same conditions for the same period of time. At the end of the

incubation, the dissolved oxygen in both the dilution water and the mixed sample is determined by the Winkler method. The difference between the dissolved oxygen in the two samples represents the oxygen consumed in oxidation of the waste. This oxygen, reported as parts per million consumed is the biochemical oxygen demand (B.O.D.) of the waste.

Oxygen Consumed. The oxygen consumed method is a chemical procedure used to determine the amount of oxygen required for the complete oxidation of a known sample of the waste. This determination is usually done by oxidizing the waste with a known excess of either standard chromate⁽³⁹⁾ or standard permanganate⁽¹⁰⁸⁾. Upon completion of oxidation the mixture is back-titrated to determine the unused oxidant. From the data obtained the parts per million oxygen required for the oxidation are calculated. This value is known as the oxygen consumed (O.C.) or the chemical oxygen demand (C.O.D.) of the waste.

Comparison of Biochemical Oxygen Demand and Oxygen Consumed Tests. The relative merits and faults of the biochemical and chemical methods of determining oxygen demand have been discussed by workers in the field⁽³⁹⁾. The biochemical method does rely on biological oxidation similar to that in the stream. However, there are many variables involved, and even under the best

controlled conditions, the results, particularly for industrial wastes, vary considerably. The chemical method gives reproducible results, but unfortunately oxidizes some materials such as cellulose which are not oxidized to any great extent in the stream, and does not oxidize ethanol and acetate which do have quite a high biochemical demand. According to Hess⁽³⁸⁾, the chemical industry is not very happy with either test, but uses them both in absence of anything better.

Industrial Waste Disposal. As has been discussed previously, industrial wastes are extremely diverse and as a group are potential stream pollutants. The existence of governmental laws and rules for the prevention and control of pollution has also been mentioned. Therefore, an industry is frequently faced with the problem of making corrective steps in its waste disposal problem.

The corrective steps which an industry may investigate can only be given in very general terms, since the wastes involved are so diverse in nature. Hess⁽³⁸⁾ in his review of wastes from chemical industries lists the following possible solutions: by-product recovery, modification of process, waste treatment, diversion to municipal sewers, and controlled dilution in receiving waters. Cecil⁽¹⁸⁾ suggests an additional possibility in the

underground disposal of process waste water. A brief consideration of these various plans follows:

By-product Recovery. As Hess⁽³⁸⁾ points out, the chemical industry has a sound scientific basis, and is designed for maximum yields with a minimum of waste. Useful by-products have usually been discovered before a plant is constructed, and means for their recovery are installed with the plant. The effluent of a modern chemical plant is usually a worthless waste, and if it contains valuable materials, they are too dilute to make recovery feasible.

Modification of Process. The possibility of modifying a chemical process to cut down on wastes is an intriguing one and is being continually exploited. Some industries have found considerable help in this plan.

Treatment of Wastes. The treatment of wastes to render them nonobjectionable before discharge is an operation that is a financial liability, with no hope of any return. However, this procedure is frequently the cheapest in the long run⁽³⁸⁾ and has been adopted by a great many industries. In many cases it does represent the only solution. Methods of treatment vary with the

waste involved and may be anything from simple neutralization or filtration to a series of complex chemical and biochemical processes. In general, organic wastes are usually treated by trickling filters, the activated sludge process, cascade channels, anaerobic digestion, or various chemical treatments of which chlorination is one of the commonest. Inorganic wastes are sometimes treated by biochemical methods but more frequently by chemical methods. Most methods used are merely adaptations of sewage treatments, and a good deal of research and design needs to be done for the treatment of industrial wastes.

Diversion to Municipal Sewers. The method of diversion to municipal sewers is not as simple as it sounds. The complexity and cost of sewer construction for industrial installations, and sewer taxes and treatment charges made by the municipality frequently make this the most expensive means of waste disposal.

Controlled Dilution in Receiving Waters. The dilution method consists primarily of impounding wastes so that low waste discharge is used during low stream flow, and the collected wastes are discharged rapidly during flood periods. The method is frequently practiced and, if done intelligently,

is quite successful. It does have a basic requirement that considerable tracts of land are available for impounding the wastes. This land requirement makes the method impractical in congested areas.

Underground Disposal of Process Waste Waters. The underground disposal method as reviewed by Cecil⁽¹⁸⁾ has been used successfully by a number of industries, particularly the petroleum industry. The method consists essentially of drilling a well into some porous stratum from which the fresh water stratum cannot possibly be polluted, and then pumping the waste into this well. The stratum selected must be porous enough to take the waste, and the waste itself must be pre-treated to prevent clogging of the disposal channels.

Biological Aspects

The biological aspects of the industrial waste problem occur principally in the biochemical treatment of the waste and in the effect of the waste on the biological population in the stream. The biological aspects of waste treatment is a rather specialized field, and considerable information is available in sanitary engineering journals and works, and it will not be considered in

this review. The effects of wastes on the normal biological population of the stream will be considered briefly in this section.

The Effects on the Macrobiological Population. The effects of wastes on the macrobiological population varies with the waste in question⁽⁶⁰⁾. Toxic materials, of course, have an adverse effect depending somewhat on the toxic substance present. Solids that tend to settle are frequently harmful to shell fish and also interfere with bottom feeding fish. Silty materials will clog the gills of most higher water animals and cause death from suffocation. Organic materials are harmful principally in that their microbiological oxidation causes depletion of the dissolved oxygen and none of the higher plants and animals can live in the complete absence of oxygen⁽⁵⁹⁾. Fish in general are believed to require water with about five parts per million dissolved oxygen⁽¹²⁴⁾, though Phelps⁽⁵⁹⁾ reports having seen carp survive, by gulping air, in water showing zero oxygen. With respect to organic pollutants, however, it should be remembered that they are basically foods, and are beneficial at low concentrations wherein oxygen is not depleted.

Effects on the Microbiological Population. The effect of wastes on the microbiological population is somewhat different to the effect on the macrobiological population. Toxic materials

will, of course, kill these organisms as well as the macro-organisms, but some bacteria, protozoa and algae are resistant to such toxic substances as hydrogen sulfide⁽⁴⁴⁾. Solids as a general rule do not bother these organisms, with the exception that an inert sludge might cover and kill some of the bottom dwellers. Organic materials probably have the greatest effect of any wastes upon the microbiological population of the stream. As is mentioned by Porter⁽⁸⁵⁾, many workers believe that microorganisms exist that are capable of attacking every known carbon compound. Whether or not this is so, it is certain that almost any organic compound can be considered as a potential food. In the presence of the food the microorganisms begin to multiply rapidly and exhibit a very rapid metabolism. If the waste concentration is high enough a pollution zone, called the zone of degradation⁽²⁾ appears. The dissolved oxygen is reduced to about 40 per cent saturation and algae are dead or dying. The population consists principally of bacteria, protozoa, and worms. If the waste concentration were sufficiently high, a second pollution zone occurs down stream from the first. This zone is called the zone of active decomposition⁽²⁾ or the septic zone⁽⁴⁴⁾. In this zone the bacteria, particularly the anaerobic and facultative forms, reach their peak population. The dissolved

oxygen is usually reduced to zero. All macrobiological forms are either absent or dead, and all obligately aerobic microorganisms are either dead or inactive. Chemically, the zone is one of extremely active decomposition of the organic materials, with methane, hydrogen, nitrogen, hydrogen sulfide and possibly carbon dioxide being the products of decomposition. This zone will continue as long as the concentration of organics remains sufficiently high to sustain the active state of growth and metabolism. As soon as the organic materials are exhausted a new pollution zone, called the zone of recovery^(2,44), appears. In this zone the microbiological population of the septic zone is on the decrease, and algae, aerobic microorganisms and some macroorganisms reappear. The oxygen content of the stream runs about 40 per cent saturation, and nitrates, sulfates and carbonates are found in the water. In this zone the stream is beginning to approach normality rapidly. The last zone of the pollution cycle is called the zone of cleaner water⁽²⁾ or the clean water zone⁽⁴⁴⁾. In this zone the stream is back to normal, the dissolved oxygen concentration approaches saturation, and the microbiological population of bacteria, protozoa, and algae is about the same as it was above the sight of initial pollution.

Water Bacteriology. The bacteria found in natural waters may be classified into three groups as follows⁽¹²⁹⁾: the natural water bacteria, soil bacteria, and sewage bacteria. Sewage bacteria are found only in waters polluted with sewage, and though this condition is fairly common, these organisms (Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Clostridium welchii, and Clostridium sporogenes) cannot be considered to be part of the normal flora. The soil bacteria are washed into waterways during rains and could be considered more or less natural inhabitants. The commonest of the soil bacteria found in water are members of the genus Bacillus. The natural water organisms that are found in river water under most conditions are members of the following genera: Pseudomonas (particularly Pseudomonas fluorescens), Chromobacterium, Achromobacter, Micrococcus, and Sarcina.

The organisms which are active in conditions of waste pollution will depend largely upon the waste. In the case of sewage pollution the sewage organisms will undoubtedly be present in large numbers in the pollution zone. However, in the case of many industrial wastes, the oxidation and dissimilation of the waste will be performed entirely by the normal stream organisms, and by the soil organisms when present. With present knowledge

it is impossible to state what the predominant species of organisms will be in any particular case of industrial waste pollution.

Pseudomonas Fluorescens. Pseudomonas fluorescens is a natural water organism⁽¹²⁸⁾, highly proteolytic in nature and possessing very weak fermentive power for most carbohydrates⁽¹²⁹⁾. Many types of this organism are known, and most types are extremely aerobic in nature. It has considerable ability to perform aerobic oxidation of many carbon compounds including hydrocarbons⁽¹⁰⁰⁾. It is one water organism that would be active in using dissolved oxygen for the purpose of oxidizing organic industrial wastes, and should be of primary importance in a fundamental investigation of the biological oxidation of industrial wastes.

Bacterial Respiration

A study of bacterial respiration involves a study of the chemistry and physics of the processes by which the organisms derive energy from the breakdown of their food materials. The various physical, chemical, and biological factors which affect the processes of obtaining energy are also of considerable importance in this field. In this section a brief discussion of bacteriology, the chemical activities of bacteria, and the mechanisms of respiration will be given.

Bacteria. Bacteria are single celled, achlorophyllous plants belonging to the class Schizomycetes^(14,90). Morphologically the cells occur as either spheres, rods, or spirals of a size ranging from 0.5 to 3.0 microns^(13,14,91). Bacterial cells are differentiated from all other cells in that they possess no optically demonstrable nucleus. Bacteria multiply by simple cellular fission, and for a given culture, a plot of the logarithm of the number of viable cells versus culture age will give a bell shaped curve similar to that shown in Figure 1^(63,64,65). The physiological activity of bacteria vary considerably in their various phases of growth. Most respirational studies are made on cultures which are approximately midway in the logarithmic phase (Line C - D, Figure 1) of growth.

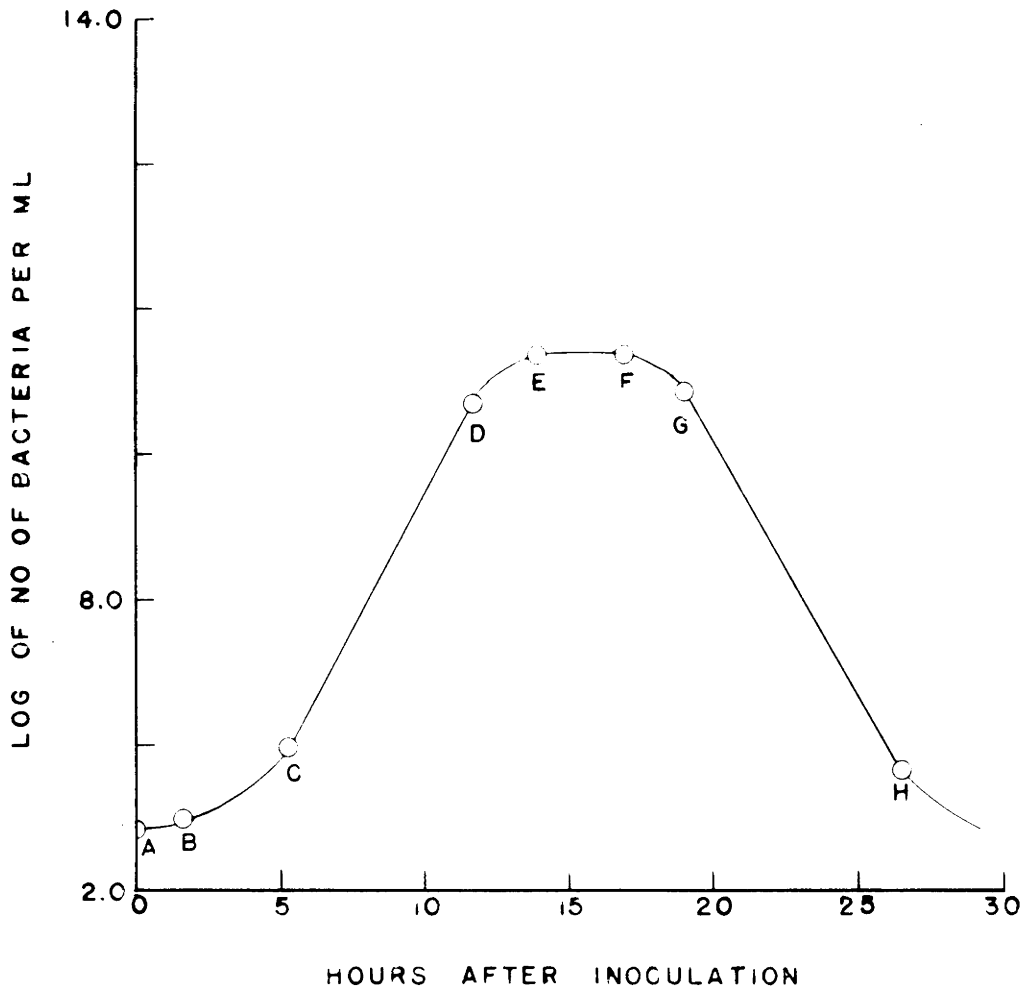


FIGURE 1. BACTERIAL GROWTH CURVE

PORTER, J. R.: "BACTERIAL CHEMISTRY AND PHYSIOLOGY,"
P. 105. JOHN WILEY AND SONS, INC., NEW YORK, N. Y.,
1946.

Effect of Physical Environment on Bacteria. Bacteria, like all other life forms, are affected by their physical environment. For practical purposes, the most important physical factors are electromagnetic radiation, temperature, and the osmotic pressure of the growth medium. For the purpose of respirational studies these physical factors should be controlled. Ultraviolet radiation is bacteriocidal and should be excluded. For maximum respirational activity tests should be made at a temperature that is experimentally determined to be the optimum, while the osmotic pressure of the medium should be the same as or very close to the same as the normal osmotic pressure of the cells. The various effects of these factors are adequately discussed in the literature^(66,67,69).

The Effects of Chemical Agents on Bacteria. Chemical agents other than normal foodstuffs frequently have harmful effects on bacteria. For optimum physiological activity chemicals which are strong oxidizing agents⁽⁷⁰⁾, protein coagulants^(71,72,73,75,77), enzyme inhibitors⁽⁷⁴⁾, or known antibiotics⁽⁷⁸⁾ should be carefully excluded from the growth or respirational medium.

Bacterial Nutrition. The nutritional requirements of bacteria vary from those bacteria which can survive in a simple inorganic medium to those which are obligately parasitic. However, in terms of the elemental requirements, most bacteria will

require the presence of acceptable sources of carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, calcium, iron, potassium, and magnesium. There is a definite possibility that traces of other elements may be required for certain specific bacteria. The specific functions of some of the required elements have been discussed in the literature^(80,81,82,84).

Chemical Activity of Bacteria. In this review the term respiration will be used to denote any chemical reactions performed by the cells on food substances for the purpose of obtaining energy. Besides acting on foods, cells are also constantly breaking down portions of their own protoplasmic components, and small amounts of energy are obtained from this source. This phenomenon is termed catabolism. The exact reasons for catabolism are not known, but since it seems doubtful that its primary purpose is that of obtaining energy, it will not be considered as a respirational mechanism. The third important chemical activity of the cells is anabolism or the synthesis of protoplasmic constituents. This activity requires energy and is therefore obviously not respirational.

Chemical Reactions Performed by Bacteria. Although the individual chemical reactions performed by bacteria in obtaining, preparing and oxidizing their foods are extremely numerous, they

are usually either hydrolytic, oxidative, or reductive in nature⁽⁸³⁾.

Hydrolytic Reactions Performed by Bacteria. The hydrolytic reactions performed are of a preparatory nature and usually involve the hydrolytic degradation of large molecules such as starches or proteins. It is interesting to note that hydrolytic reactions are usually extracellular in nature⁽⁷⁹⁾. It would seem then, that the principal purpose of hydrolysis is to render food substances of such molecular size that they may be taken within the cell.

Oxidation and Reduction Reactions Performed by Bacteria. Oxidation and reduction are concurrent reactions whether occurring in test tube or cell, and will be considered together. Oxidations are, in most cases, energy liberating reactions. The cells oxidize their food materials and at the same time reduce some other material such as molecular oxygen. The energy liberated in these reactions is used by the cell for other activities. Oxidation reduction reactions take place within the cell or intracellularly^(16,79) which is, of course, the point at which energy is required and used.

Types of Respiration. As has been mentioned previously, bacteria vary considerably in their oxygen requirements. Some bacteria cannot grow in the absence of molecular oxygen, and some cannot grow in the presence of molecular oxygen. Then again, some

bacteria can grow either in the presence or absence of molecular oxygen⁽¹⁵⁾. This variation in oxygen requirement can only mean that different respirational processes are involved for different bacteria, since respiration depends upon oxidation of some form. The types of respiration must depend on the substance or substances reduced. For example, glucose may be oxidized both by bacterial forms that require oxygen and those that will not grow in the presence of oxygen. Those using oxygen reduce the molecular oxygen to water, and those which do not utilize oxygen must reduce some other substance. Therefore, the various types of respiration may be defined in terms of the substances reduced, or the hydrogen acceptors⁽¹⁵⁾ as they are frequently called in biological work.

Aerobic Respiration. Bacteria which require molecular oxygen for their respiration purposes, and will not grow in the absence of free oxygen are called strict aerobes^(15,82). Some bacteria require oxygen for their respirational activities but in the absence of free oxygen are able to obtain their oxygen from some other substance such as nitrates, which is present in their environment. These bacteria are called facultative aerobes⁽⁹⁴⁾. It follows then, that aerobic respiration can be defined as any respirational process in which oxygen is the substance reduced or the hydrogen acceptor⁽¹⁵⁾.

Anaerobic Respiration. Bacteria that cannot grow in the presence of molecular oxygen are called strict anaerobes^(15,82). Bacteria which can live in the presence or absence of oxygen, but do not utilize oxygen are called facultative anaerobes⁽⁹⁴⁾. In both cases, some substance other than free oxygen is being reduced, and anaerobic respiration is defined as any respiratory process in which something other than oxygen is the substance reduced or the hydrogen acceptor⁽¹⁵⁾.

Microaerophilic Bacteria. Although many typical examples of the above mentioned types of respiration can be found, there are some bacteria that are intermediate types. These types are inhibited by oxygen in atmospheric concentrations, yet will not grow in a medium totally devoid of dissolved free oxygen⁽⁸²⁾. These organisms are placed in a separate group and are said to be microaerophilic.

Popular Misconception of Respiration. It should be noted that aerobic and anaerobic respiration are sometimes defined as life in the presence of air and life in the absence of air. It can readily be seen that these definitions would lead to considerable confusion, particularly in the case of facultative organisms. These definitions should therefore be avoided.

Mechanism of Respiration. Respiration is a process of oxidative degradation of the metabolites involved. In complete aerobic oxidation of a carbohydrate such as glucose the final products would be carbon dioxide and water. Glucose can be oxidized by simple combustion in the laboratory but it must, in the absence of catalysts, be heated to a temperature in excess of 200 °C. Since 98 °C is the upper limit at which any living body can metabolize⁽⁶⁷⁾ this reaction is not possible biologically. Glucose can also be oxidized at lower temperatures by the use of certain catalysts and strong oxidizing agents. However, these conditions are all too severe to occur in the living cell. It follows then, that living cells must possess some mechanism for the oxidation of foodstuffs which will operate under the very restricted conditions necessary for life. It seems obvious that such conditions would require very special catalysts.

Properties of Enzymes. As a result of much painstaking work it has been clearly demonstrated that the cells do indeed utilize very special catalysts in performing their many chemical reactions. These catalysts are called enzymes. There are a great many enzymes known, and all those thus far studied have certain common properties. Some of the important common properties are as follows^(101,103): first, they are highly specific in their action. Second, they are organic catalysts produced by living cells.

Third, they catalyze reactions that are essentially equilibrium reactions and do not initiate reactions. Fourth, they are usually proteins of rather high molecular weight, 20,000 or above. Fifth, they are usually inactivated by any conditions which would be harmful or lethal to the life form from which they were obtained. Sixth and last, all enzymes require the presence of water and some electrolyte to be active.

Action of Enzymes. The usually accepted theory of enzyme action postulates the formation of an intermediate compound composed of the enzyme and the substance acted upon. The substance acted upon is usually called the substrate. The enzyme-substrate complex breaks down in turn to yield the products of the reaction plus the enzyme⁽¹⁰¹⁾. The enzyme is, of course, used over and over again. This type of reaction has been proven for some enzymes, while for others, definite experimental proof of the mechanism of reaction is lacking⁽¹⁰³⁾. A few enzyme reactions are believed to be chain reactions⁽¹⁰¹⁾. In this type of reaction the enzyme activates one molecule of the substrate. This activated molecule will, upon collision with a second substrate molecule, decompose, and at the same time activate the second molecule. This pattern would then be repeated over and over until all substrate was decomposed. This type of chain reaction

is believed to occur with a few of the enzymes which are concerned with oxidation reduction reactions.

A great deal has been written about the kinetics of enzyme reactions⁽¹⁰¹⁾, and in some instances they do seem to obey, at least in part, some of the physicochemical laws⁽¹⁰³⁾. However, it must be remembered that enzyme systems are colloidal and not simple solutions. It is not surprising then that enzymatic reactions do deviate somewhat from the theoretical, hence this situation raises some question as to the validity of interpreting these systems on the basis of the classical physicochemical laws.

Classification of Enzymes. Enzymes are named under the four following systems of nomenclature⁽¹⁰¹⁾: substituting or adding the ending -ase on the root stem of the name of the substrate being decomposed. For example, the enzyme which decomposes maltose is called maltase and that which decomposes urea is called urease. A second system involves combining the ending -ase with the name of one of the products of reaction, for example, alcoholase for the enzyme responsible for the formation of alcohol. A third system consists of combining the ending -ase with the name of the reaction taking place, as oxidase or hydrolase. The last system is a system of priority in which

classical names given by early workers are used, such as rennin and pepsin.

A similar system is used for naming groups of enzymes. In this case, the enzymes are usually grouped under one of the three following systems⁽¹⁰¹⁾: first, by adding the ending -ases to the name of typical groups of chemical substrates attacked, as carbohydases and proteinases. The second system involves adding the ending -ases to the type of reaction performed such as oxidases and hydrolases. The third system consists of using the respective spatial position of the enzymes in regard to the cells from which they originate, giving two groups, intracellular and extracellular enzymes.

Enzyme Systems in Respiration. As might be guessed from the high degree of specificity and complexity of the enzymes, in general no single enzyme is responsible for the respirational oxidations performed in the cells. The complete oxidation of a simple carbohydrate such as glucose to carbon dioxide and water is extremely complex with a large number (over 20) of intermediate steps⁽¹⁰²⁾. This oxidation requires a system of enzymes, each one catalysing a particular reaction in the oxidation. Some of the enzymes present are independent and can act on their particular substrate alone or in the presence of other enzymes. Some enzymes are in dependent groups which will not function

unless all members of the group are present. Some enzymes react only in the presence of certain secondary activators or carriers called coenzymes. All enzymes require very definite conditions, such as correct pH, correct salt concentration and correct temperature range to react with their substrate.

Because this field is so specialized and complex no attempt will be made to review it further. Suffice it to say that in anaerobic respiration there are no enzyme systems present that will couple the oxidation (dehydrogenation) of the substrate with molecular oxygen. In this case, some portion of the degraded substrate is the usual hydrogen acceptor. A typical example is the degradation of glucose to alcohol and carbon dioxide:



In aerobic respiration the oxidation of the substrate is coupled enzymatically to molecular oxygen yielding water and carbon dioxide as the final products.

Oxygen Requirements of Bacteria

The rate at which bacteria will remove the dissolved oxygen from their environment depends upon the rate of consumption per cell, and the number of cells present. The cells present at any given time depends upon the multiplication rate. When the rate of utilization of oxygen in the medium exceeds the rate of diffusion of oxygen into the medium, anaerobic conditions result. In a river, anaerobic conditions give rise to the phenomena known as pollution. As far as bacteria are concerned, pollution must be a function of rate of multiplication and rate of oxygen uptake. The factors influencing the rate of oxygen uptake of bacteria will be discussed in this section.

Introduction. From the fundamentals of bacteriology certain generalizations can be made concerning bacterial oxygen requirements⁽¹⁵⁾. The anaerobic bacteria will not require any oxygen. The microaerophilic bacteria will have definite oxygen requirements, but the concentration of dissolved oxygen in their environment must be lower than that in equilibrium with atmospheric oxygen. The aerobic bacteria will require oxygen and the strict aerobes will tolerate dissolved oxygen concentrations equal to that in equilibrium with atmospheric oxygen. Many of the strict

aerobes will tolerate dissolved oxygen concentrations in equilibrium with at least one atmosphere of oxygen.

It is known that unfavorable physical or chemical environment hinders the growth rate of bacteria^(66,70). Since this hindrance of growth would cut down on the number of cells present, it is likely that the over-all oxygen requirements of a culture under these adverse conditions would be less than under ideal conditions. This information tells nothing of the oxygen requirements of the individual cells.

The number of cells present in a culture depends upon the age of the culture⁽⁶³⁾. Since the over-all oxygen requirements will depend on the number of cells, it is obvious that oxygen requirements will also be affected by the age of the culture.

In mixed cultures, the relationship of the organisms present could affect oxygen demands. In the case of symbiosis or commensualism⁽⁹⁵⁾ the total number of cells would probably be increased over normal and might result in increased oxygen requirements. In the case of antibiosis the number of cells would be decreased, and oxygen demand would probably be affected. In synergism, where a new chemical activity is being performed as a result of the joint effort of the organisms present, it is quite possible that oxygen demands may be affected.

In summary it can be stated that the oxygen requirements of a culture will be affected by the kind or kinds of bacteria present, the environment of the bacteria, and the age of the culture. To make any statement concerning the direction and magnitude of said effect in a definite case, specific information must be obtained either from the literature or from laboratory experiment. Some of the fragmentary information present in the literature will be presented herewith.

Methods of Determination of Oxygen Requirements. To determine the oxygen requirements of bacteria, the general method is to measure the amount of oxygen used by a given quantity of organisms in a definite length of time. In the case of a closed system, the oxygen removed from the gaseous phase and used by the organisms can be measured by the standard methods of oxygen determination⁽²⁶⁾. This method involves rather large liquid and gas volumes, and it is quite time-consuming. A second method consists of determining the dissolved oxygen content of a medium before and after some organism has been respiring therein. The liquid is, of course, kept out of contact with the air during culture. This method is an adaptation of the standard "biochemical oxygen demand" determination^(107,109). It also has the disadvantage of being very time-consuming and gives only terminal results. Both of these methods utilize a chemical determination of

oxygen. However, if oxygen is known to be the only gas being transferred from the gaseous phase, the transfer will, in a closed system held at constant volume, cause a pressure decrease. In this case the pressure decrease will be directly proportional to the oxygen used. This fact is utilized in a piece of equipment known as the "Warburg micro-respirometer," or "Warburg manometer."

Warburg Manometric Equipment. The Warburg manometer is a standard "U" shaped manometer. It is constructed of capillary glass tubing approximately one millimeter in bore and seven millimeters in outside diameter. The manometer is about 40 centimeters high and has a graduated section on each leg 30 centimeters long. The unit graduation on the manometer is one millimeter. One leg of the manometer, normally the left leg, is open to the atmosphere. The other leg is equipped with a tee. On the run of the tee is a glass stopcock by which this leg may be opened to or closed from the atmosphere. The side outlet of the tee is connected to a capillary glass lead (of the same dimensions as the manometer) which runs about 8 centimeters in a direction perpendicular to the plane of the "U" and then turns vertically downward and terminates in a standard taper male ground glass joint about 8 centimeters below the bend. This joint is used to connect the reactor flask to the manometer. At the bottom of the "U" of the manometer is a take-off line about 15 millimeters

long, also constructed of a capillary glass. This take-off is connected to a tygon or rubber sac. The sac acts as a reservoir of manometer fluid, and the liquid in the manometer may be raised or lowered by means of a screw clamp attached to the reservoir⁽¹¹¹⁾.

The reactor flask consists of an approximately 15-milliliter conical flask with a standard taper female ground glass joint serving as the mouth of the vessel. It may have one or more side arms which are usually joined to it at a point near the neck of the flask. These side arms have a volume of approximately two milliliters. The flask is also equipped with a small central well of one or two milliliters capacity^(111,115).

A sketch of the complete assembly of a Warburg manometer and its reactor flask is shown in Figure 2.

In actual operation the flask is completely immersed in a constant temperature bath and flask and manometer are jointly subjected to mechanical agitation. The agitation increases the liquid-gas interface and provides complete mixing. This increases the rate of diffusion of gas into or out of the liquid⁽¹¹³⁾.

In practice, the substrate or metabolite solution is placed in the side arm of the flask, the cellular suspension is placed in the body of the flask, and a 20 per cent solution

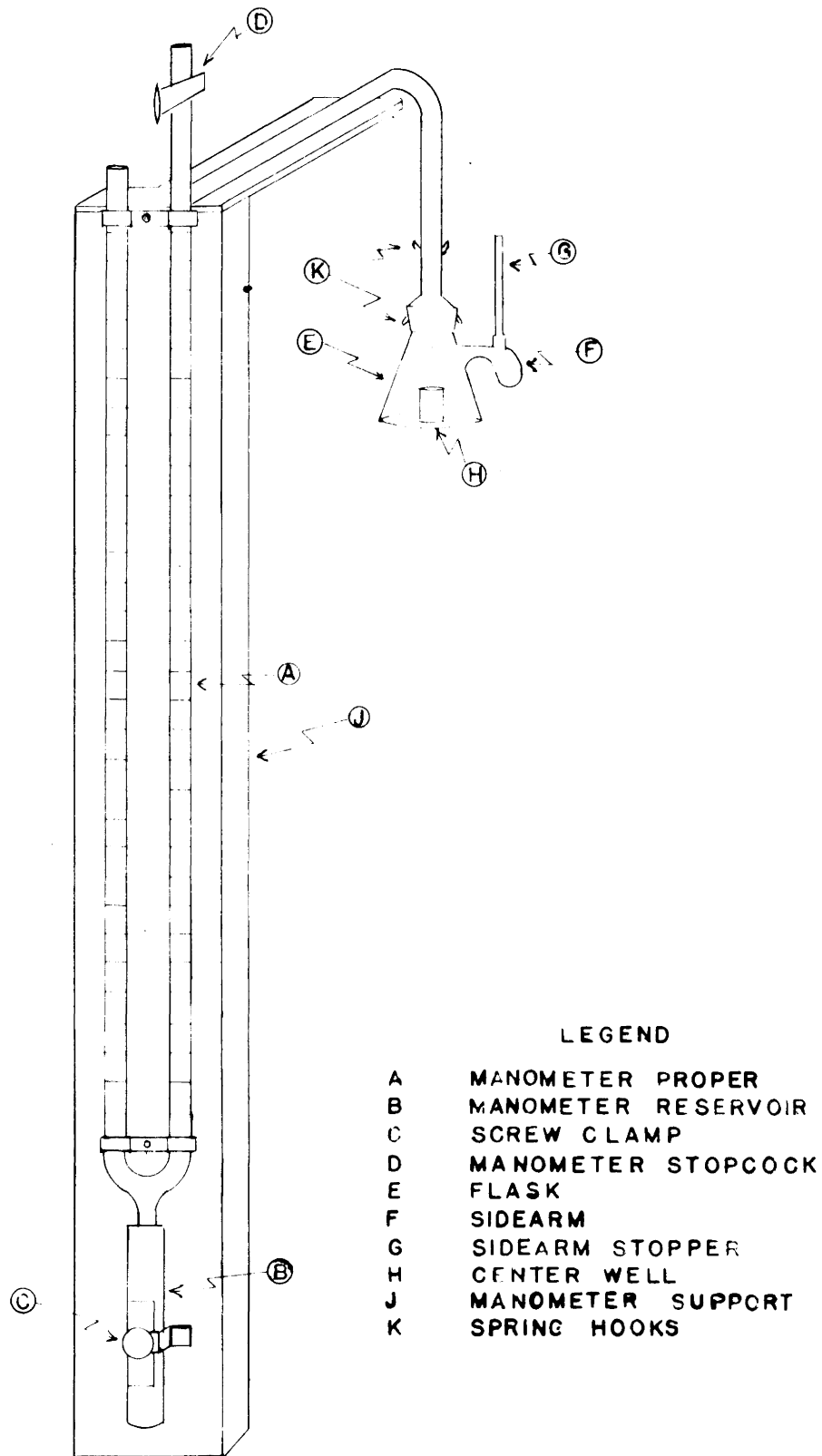


FIGURE 2. WARBURG CONSTANT VOLUME MANOMETER AND FLASK

of potassium hydroxide is placed in the center well. The cellular suspension and metabolite may be mixed at any time by simply tipping the flask. The potassium hydroxide in the center well is used to absorb any carbon dioxide which may be given off, so that oxygen is the only gas involved in the exchange between the liquid suspension and the gas phase⁽¹¹¹⁾.

Resting Cell Technique. If oxygen consumption tests are made with growing cultures of bacteria, it is difficult to express the rate of uptake as a function of the changing bacterial concentration. For this reason, workers in the field of bacterial respiration have long used a technique called "resting cell technique." In essence, this involves suspending the cells, which have been washed free of their growth medium, in a buffered medium which is so constituted as to permit respiration but not growth or multiplication. The technique has been adequately described in the literature^(34,56,113,118,119). Cells to be used in resting cell technique studies are usually grown at optimum temperatures in the most favorable medium. Carbohydrates are excluded from the growth medium if their presence enhances formation of gums and capsular material which would serve as reserve food for the cells. Cells are generally removed from their growth medium by centrifugation, and are washed, with agitation either in distilled water or buffer. If suspensions of the same cellular

concentration are needed for a series of tests, nephelometric methods offer a quick means of suspension analysis. In determination of oxygen consumption of resting cells, a blank consisting of cells suspended in buffer is always tested. The oxygen consumption of the blank is called the endogenous rate. Whether or not the endogenous rate should be subtracted from the rate of oxygen consumption in presence of substrate is a matter of question that has not yet been satisfactorily answered. There is the definite possibility that endogenous rate may be either suppressed or enhanced in the presence of substrate, and that subtraction of endogenous rate from total rate of oxygen uptake does not give the rate of uptake caused by the substrate. However, in most cases, subtraction gives the only available approximation of rate of oxygen consumption caused by substrate.

Effect of Temperature on Oxygen Requirements of Bacteria.

Temperatures above and below the range in which bacteria are able to metabolize will, of course, reduce the oxygen requirements of any bacterial system to zero. Some work on the effects of temperature changes within the range in which bacteria are active indicate that temperature has a definite effect on oxygen demand. Deotto⁽²³⁾ found that cells of Escherichia coli, when exposed to a temperature of -2 to -3 °C for 20 to 40 minutes

consumed 200 to 500 per cent more oxygen than controls held at 38 °C. The author postulated the possible liberation at low temperatures of some substance that greatly stimulated respiration. This theory is not impossible, but it should also be kept in mind that at temperatures just below freezing it is possible for ice crystals to grow within the cells and thereby rupture them. The rupture of the cells would result in the liberation of the intracellular respirational enzymes which might also be at least partially responsible for the increased oxygen uptake. Zobell⁽¹²⁰⁾ working with a mixed culture of lake water bacteria found that the rate of oxygen consumption increased between 8 and 25 °C, but that an increase to 37 °C injured some of the bacteria present and resulted in a lower rate of uptake for the mixture. Stern and Frazier⁽⁹⁹⁾ performed a series of experiments on the oxygen uptake of Lactobacillus bulgaricus. They reported that the rate of oxygen consumption during the first 30 minutes of their tests was greater at 49.5 °C than at 37 °C. However, after this time elapses, the rate at 49.5 °C dropped rapidly while that at 37 °C remained constant. This may have been caused by inactivation of the enzymes at the elevated temperature, a phenomenon quite common in enzymatic investigations⁽¹⁰¹⁾.

Effect of Light on Bacterial Oxygen Consumption. It has been known for many years that light definitely affects bacteria, and that certain wave lengths in the ultra-violet region are definitely lethal. However, the effect of light on oxygen consumption has not been studied extensively. Rubenstein⁽⁸⁹⁾ working with Sarcina lutea found that the rate of oxygen consumption of this organism at 37 °C is decreased by exposure to light from a common Mazda lamp. Since the natural habitats of this organism are given in Bergey's Manual⁽⁹⁾ as air, soil, water, and skin surfaces, it seems logical to assume that this type of radiation would be nonlethal. However, it is possible that the radiation may have some direct effect on the respiratory mechanism.

Effect of Oxygen Availability on Oxygen Consumption. As might be suspected, the availability of oxygen, and the concentration of dissolved oxygen in a medium affects the oxygen consumption of bacteria. Zobell⁽¹²²⁾ working with a mixed culture of lake water bacteria found that while bacterial multiplication was definitely influenced by the concentration of dissolved oxygen in lake water, respiration for a constant number of cells remained independent of concentration between 0.3 and 26.48 milligrams of oxygen per liter of media. Concentrations below 0.3 milligram per liter resulted in a marked decrease in respiration. Hershey⁽³⁷⁾ working with Escherichia coli reported that

under cultural conditions, rates of growth and respiration are both limited by the rate at which oxygen can reach the cells. He also noted that if the rate at which oxygen is made available is increased, the rate of oxidation of foodstuffs is greatly increased. Rahn and Richardson^(86,87) working with several organisms, and Schlayer⁽⁹⁶⁾ working with type I pneumococci (Diplococcus pneumoniae, Type I)⁽¹⁰⁾ reported that growth and respirational rates depend on availability of oxygen. From these studies it is obvious that oxygen must be made available at some rate greater than that at which it is being used. If the rate of availability is less than the rate of use, the oxygen consumption will depend on the rate of diffusion rather than on the rate of consumption.

Effect of pH on Bacterial Oxygen Consumption. The reaction or pH of a medium is known to affect bacterial growth and metabolism⁽⁷¹⁾, and can in extreme cases cause a complete cessation of these activities. It seems quite logical that pH might affect oxygen consumption or respiration, also. Thorne and Walker⁽¹¹⁰⁾ in their physiological studies of Rhizobium investigated this possibility. They found, in the case of Rhizobium japonicum and Rhizobium meliloti that the maximum oxygen uptake for both these organisms was in the range of pH 7.2 to 8.0. They also observed that oxygen consumption, though very slow, was

detectable in both alkaline and acid solutions in which growth had ceased, indicating that the respirational pH range is greater than the growth pH range. It is also interesting to note that, according to the results of these investigators, the optimum pH values for oxygen uptake did not coincide with the optimum for growth, which were pH 7.0 for Rhizobium meliloti and pH 6.7 to 6.9 for Rhizobium japonicum.

Effect of Inorganic Salts on Oxygen Consumption of Bacteria.

In the natural habitats of bacteria there is always some concentration of inorganic salts. Since inorganic salts are known to affect various life processes, it seems possible that oxygen consumption might be affected by their presence. Many studies have been made along this line. McLean and Fisher⁽⁴⁷⁾ reported that Serratia marcescens required considerably more oxygen when it was assimilating inorganic ammonia than for other nitrogen sources. These workers calculated that 2.2 oxygen atoms were taken up for each ammonia nitrogen assimilated. Mickelson and Shideman⁽⁵²⁾ found that in the oxidation of glycerol by Escherichia freundii addition of phosphate ion increased both oxygen uptake and glycerol consumption, with the optimum phosphate concentration being 0.1 molar. Since they also found that adenosine triphosphate was about 2.5 times as effective as inorganic phosphate the effect is probably caused by phosphorylation

of intermediates in the glycerol dissimilation. Perlman⁽⁵⁸⁾ found that the absence of some metallic ions, particularly magnesium and chromium, affected a redistribution of the fermentation products of glucose by Aerobacter aerogenes. The report on chromium is rather unusual since it is normally considered to be toxic. Brooks⁽¹²⁾ found that magnesium concentrations between 0.01 and 0.03 molar did not affect the respiration of Bacillus subtilis, but that concentrations above 0.03 molar caused a decrease in the rate of respiration. Ingram⁽⁴⁰⁾, working with Bacillus cereus, found that the chlorides, sulfates, acetates, and nitrates of sodium, potassium, magnesium, calcium lithium, ammonium, and cerium increased respiration in low concentrations and decreased it at higher concentrations. In view of all of these facts it may be assumed that in general the inorganic salt composition and concentration of a medium might affect the oxygen consumption of the bacteria contained therein.

Effect of Bacterial Culture Age on Oxygen Consumption. Many workers in the field of bacteriology have found that cultural age affects many of the physiological activities of bacteria, the rate of oxygen consumption being amongst these activities. Greig and Hoogerheide⁽³⁴⁾ reported that the oxygen uptake of a culture is proportional to the bacterial content when the content is determined nephelometrically. They found this relation to be

valid for Proteus vulgaris, Staphylococcus aureus, (Micrococcus aureus, Var. pyogenes)⁽⁷⁾ Pseudomonas fluorescens and the yeast Willa anomala. Walker and Winslow⁽¹¹⁸⁾ working with Escherichia coli found that the activity of the cells was greatest during the period of youth, declined somewhat during the logarithmic phase, and showed the least activity during the stable phase. Clifton and Logan⁽²¹⁾ working with the same organism also reported that the age influenced the oxygen consumption per cell hour. Clifton⁽²⁰⁾ found the rate of metabolism per cell to be highest during the early phases of growth for the three organisms: Elberthella typhi, (Salmonella typhosa)⁽¹¹⁾ Aerobacter aerogenes and Escherichia coli. From these data it can be concluded that the cultural rate of oxygen consumption increases as the number of cells present increase, and that the rate of oxygen consumption per cell is highest early in the growth of the culture, and has a lesser value at any time thereafter.

Effect of Carbon Source of Energy on Bacterial Oxygen Consumption. In the final analysis, most of the oxygen taken up by bacteria is used by them to oxidize some compound for the purpose of obtaining energy. Of course, some organisms are capable of oxidizing some compounds in the complete absence of oxygen, but many require oxygen to oxidize some, if not all compounds that they can attack. Niven, Evans and White⁽⁵⁵⁾ found

that members of the genus *Streptococcus* require oxygen for the oxidation of butyric acid. Gunsalus and Sherman⁽³⁵⁾ found that members of this same genus require oxygen to produce acid from glycerol, though they would attack glucose in the absence of oxygen. Colwell⁽²²⁾ reported that the Staphylococci (now listed as Micrococci in Bergey)⁽⁶⁾ can attack mannitol only in the presence of oxygen. Randles and Birkeland⁽⁸⁸⁾ observed that both Escherichia coli and Aerobacter aerogenes, which can attack glucose in the absence of oxygen, require oxygen to attack malate or fumarate. They did observe, however, that Escherichia coli could, in 48 to 72 hours, attack fumarate anaerobically in the presence of formate.

Since it has been established that some bacteria require oxygen to oxidize certain compounds, the next question that arises is does a given type of bacterium require oxygen at the same rate for all compounds that it can oxidize, or does it require oxygen at different rates for different compounds. This problem has been studied both directly and indirectly by several workers.

Bacterial Oxygen Consumption on Carbohydrates and Organic Acids. Carbohydrates and organic acids are both constituents of wastes arising from the chemical processing of wood and wood derivatives⁽⁴³⁾. The oxygen consumption of bacteria utilizing

these classes of chemicals are pertinent to the problem at hand. Some of the more interesting results given in the literature will be reviewed herewith.

Steinbach⁽⁴⁸⁾ studied the respiration of a number of species of bacteria using glucose, lactate, fumarate, pyruvate, succinate, citrate, and some amino acids as the compounds to be oxidized. He found that oxygen uptake varied considerably with these different compounds. Jazeski et al.⁽⁴¹⁾ reported a study of the action of Mycobacterium phlei, a micrococcus, and two cultures of the genus Pseudomonas on various fats, fatty acids, and fatty acid esters. Widely different rates of oxygen consumption were observed both between organisms on the same substrate and with the same organism on different substrates. It was also demonstrated that oxygen uptake on sodium salts of the fatty acids was in no way comparable to the oxygen consumption on methyl and ethyl esters of the same acids. For one of the species of Pseudomonas tested it was noted that sodium acetate reduced the rate of oxygen consumption below that of the endogenous rate.

The organism Mycobacterium tuberculosis has been shown to vary in its rate of oxygen uptake depending on the compound it is oxidizing. Loebel, Sherr and Richardson⁽⁴⁶⁾ made an extensive study of its respiration on 12 compounds of known structure and 5 biological materials of unknown composition. The experiments

were carried out in the Warburg micro-respirometer using the so-called "resting cell technique." All results on the oxygen uptake caused by the various compounds were compared with glycerol using the following equation:

$$\frac{\text{Respiration in compound} - \text{Endogenous rate}}{\text{Respiration in 5 per cent glycerol} - \text{Endogenous Respiration}} \times 100$$

This equation would, of course, give the percentage of oxygen uptake compared to glycerol, assuming that endogenous rate may be subtracted. Since readings are taken over the same time interval, it likewise gives a rate comparison factor. Some of the results using this method of reporting were as follows: glucose, five tests, 35 per cent, 29 per cent, 0 per cent, 0 per cent, and 33 per cent; levulose, three tests, 9 per cent, 0 per cent, 0 per cent; glycogen, two tests, 16 per cent and 29 per cent; sodium palmitate, two tests, 157 per cent and 197 per cent. The authors ran many other tests and comparisons, but these suffice to show the considerable variation in oxygen uptake with various foodstuffs. Bernheim⁽⁴⁾ in a later study extended this work, and verified most of the above findings. He stated that carbohydrates, amino acids, and other acids such as lactic, citric, and succinic have little effect on the rate of oxygen uptake. All aldehydes were found to increase the uptake, as were

the sodium salts of the lower fatty acids and alcohols (except ethyl and capryl). It was also observed that the sodium salts of salicylic and benzoic acids markedly increased the uptake. These data show that the rate of oxygen uptake of Mycobacterium tuberculosis is affected by substrate and also that certain homologous series of compounds such as aldehydes and alcohols have a similar effect on oxygen consumption.

One of the most complete studies of oxygen uptake on various foodstuffs was that made by Nunheimer and Fabian⁽⁵⁶⁾ in their work on the respiration of Micrococci. Their work covered five species of Micrococci and 35 different chemicals of known composition. The data were determined in the Warburg micro-respirometer by means of resting cell technique. Optimum conditions for respiration were determined and used in each test. The quantity of foodstuff added in each case was one milliliter of 1/30 molar solution. Their results are reported as $Q(O_2)_N$ or the microliters of oxygen consumed per hour per milligram of bacterial nitrogen. The values obtained for some of the commoner foodstuffs are given in Table II. These data give clear warning of the danger of making broad generalizations concerning the rate of oxygen uptake of bacteria on a given compound. The organisms used are closely related forms, yet there is a wide difference

TABLE II

The $Q_{(O_2)}^{N^x}$ of Genus Micrococcus on
Various Substrates

Compound	M. luteus	M. flavus	M. aurantiacus	M. cinnamonaceus	M. freundreichii
d-Arabinose	36.5	27.7	31.3	63.0	56.5
l-Arabinose	39.1	40.4	30.3	64.1	55.9
d-Xylose	56.5	130.3	32.5	74.3	65.4
d-Glucose	145.7	79.5	141.7	318.7	204.0
d-Fructose	67.9	40.7	99.4	465.8	74.1
Maltose	145.8	193.4	41.1	371.5	180.0
Sucrose	201.8	143.2	390.4	421.2	164.1
Ethanol	150.0	194.8	333.1	406.7	552.5
Glycerol	46.3	151.4	279.6	378.5	230.1
Formate	43.0	55.0	127.8	207.9	34.1
Acetate	141.6	170.7	57.0	511.7	32.3
Endogenous	41.8	23.1	16.2	38.1	31.5

$^x Q_{(O_2)}^N$ = The microliters of oxygen per hour per milligram of bacterial nitrogen

Nunheimer, F. D. and F. W. Fabian: Respiratory Studies of Micrococci, J. Bact. 44, 215-232 (1942).

in rate of oxygen consumption between organisms on any given compound, and between compounds with a given organism.

Randles⁽¹²⁷⁾ reports a very interesting series of experiments on the oxidation of fatty acids by Neisseria catarrhalis. Acids tested were straight chain fatty acids from acetate through laurate with the exception of the 9 and 11 carbon acids. The initial rate of oxygen consumption on all acids was approximately the same, with the exception of propionate, which is oxidized at a slower rate than the others. However, all acids with an odd number of carbon atoms showed a rate of oxygen consumption approximating that of propionate after the acid had been partially oxidized. The author interpreted this as a production of propionate during oxidation of the odd-numbered carbon fatty acids. It was also observed that the presence of acetate enhanced the oxidation of propionate, though no explanation was made for this phenomenon.

Effect of Concentration of Substrate on Bacterial Oxygen Consumption. The effect of concentration of foodstuffs on oxygen demand has been studied by several workers. Hershey and Bronfenbrenner⁽³⁶⁾ found that in the case of bacteria of intestinal origin the rate of growth, multiplication, and oxygen uptake were all influenced by the concentration of lactose in the medium. Zobell and Grant⁽¹²¹⁾ working with marine organisms, Escherichia coli, Staphylococcus citreus (Micrococcus citreus)⁽⁷⁾ Proteus vulgaris

and Bacillus megatherium reported that in solutions containing less than 10 milligrams per liter of organic matter, multiplication and oxygen consumption is approximately proportional to the concentration of foodstuffs present. This effect would appear to indicate that at low concentrations the rate of oxygen uptake is proportional to the food concentration up to a certain point, but that further concentration does not increase the rate of uptake. This observation seems logical since the same phenomenon is usually observed in enzyme chemistry⁽¹⁰³⁾.

Effect of Substrates on Each Other in Bacterial Oxygen

Consumption. One final important question concerns the possible effect of one chemical upon another that is being oxidized. This effect has not been investigated to any great extent. Fuller and Norman⁽³⁰⁾ reported that the aerobic oxidation of cellulose by certain mesophilic soil organisms was enhanced by the presence of xylan and hindered, in the case of the most active bacteria, by the presence of lignin. The action of xylan is not clear, since it is considerably attacked during the digestion. Possibly the xylan or some of the products of its dissimilation are catalytic for cellulose oxidation. The authors suggest that the effect of the lignin is simple mechanical protection of the cellulose fibers. This theory has not been verified, however.

In view of this information and the general knowledge that oxygen demand varies with various foodstuffs, and can be affected by salt concentrations, pH, and other factors, it seems fairly logical to assume that in many cases one compound might have an effect on the bacterial oxidation of some other compound.

III. EXPERIMENTAL

The experimental section includes a statement of the plan of investigation and an outline of the plan of experimentation. The materials and apparatus used are described. The methods of procedure used in the experimental tests, the data obtained in the tests, the calculated results, and sample calculations are shown.

Purpose of Investigation

The purpose of this investigation was to obtain fundamental information concerning stream pollution by studying the effect of substrate concentration and temperature of environment on the rate of oxygen utilization by a pure culture of a typical stream bacterium, Pseudomonas fluorescens. The substrates studied were glucose, xylose, arabinose, and acetate, chemicals of known structure and representative materials of wood pulp waste.

Plan of Experimentation

The plan of experimentation used in this investigation was as follows:

Literature Search. The literature was surveyed to obtain information on waste disposal, bacteriology, oxygen uptake by bacteria, and methods of measuring the bacterial oxygen uptake.

Method of Obtaining Data. The data were obtained using the Warburg method⁽¹¹²⁾ in conjunction with the resting cell technique^(34,56,119). Bacterial concentrations used in the tests were determined nephelometrically⁽¹¹⁶⁾ by means of a Klett-Summerson photoelectric colorimeter.

Determination of the Effect of Substrate Concentration. In determining the effect of substrate concentration on the oxygen uptake by Pseudomonas fluorescens the cellular concentration, the salts concentration of the environment, the pH of the environment, and the temperature of the environment were all held constant. The concentrations of the substrates were varied as follows, concentrations being increased by a factor of two per step in all cases: glucose, 10 steps from 0.0008 to 0.800 millimole per test; acetate, 10 steps from 0.0008 millimole to 0.8000 millimole per test; xylose, 4 steps from 0.100 to 0.800 millimole per

test, and an additional test of 0.600 millimole being made in this case; and arabinose, 4 steps from 0.100 to 0.800 millimole per test.

Determination of the Effect of Temperature. In determining the effect of temperature on the oxygen uptake by Pseudomonas fluorescens, the cellular concentration, the substrate concentration, the salts concentration of the environment, and the pH of the environment were all held constant. Temperatures tested were 15.0, 17.5, 20.0, 25.0, 30.0, 35.0, and 37.0 °C.

Materials

The materials used in this investigation on the oxygen uptake by Pseudomonas fluorescens were as follows:

L-Arabinose. Powder, C.P., melting point 159 °C, lot No 483078, distributed by the Fisher Scientific Co., Pittsburgh, Pennsylvania. Used for determining the rate of oxygen uptake by Pseudomonas fluorescens on arabinose.

Benzene. The benzene employed had been previously used in extraction of ethanol and isopropanol, was saturated with water and contained not more than 3 per cent mixed alcohols. Original specifications were: purified, 99-100 per cent, code 1444, lot 0240, distributed by the General Chemical Division, Allied

Chemical and Dye Corporation, New York, New York. Used as a grease solvent in cleaning Warburg flasks.

Evans Blue. Powder, manufactured by the Eastman Kodak Company, Rochester, New York. Used to color manometer fluid.

D-Glucose. Granular, dehydrated, bacto grade, control No 393621, manufactured by the Difco Laboratories, Inc., Detroit, Michigan. Used in determining the oxygen uptake by Pseudomonas fluorescens on glucose.

Lanolin. Anhydrous, purified, lot No 499143, distributed by the Fisher Scientific Company, Pittsburgh, Pennsylvania. Used in the preparation of glass joint lubricant.

Mercury. Redistilled, C.P., distributed by the Central Scientific Company, Chicago, Illinois. Used in calibration of manometric equipment.

Nutrient Agar. Powder, dehydrated, bacto grade, control No 394688, manufactured by the Difco Laboratories, Inc., Detroit, Michigan. Used in preparing slants for stock cultures.

Nutrient Broth. Powder, dehydrated, bacto grade, control No 387625, manufactured by the Difco Laboratories, Inc., Detroit, Michigan. Used as medium for growing test organisms.

Orbile. Anhydrous powder, purified, lot No 483864, distributed by the Fisher Scientific Company, Pittsburgh, Pennsylvania. Used in preparation of manometer fluid.

Petroleum Jelly. Purified, white, "Vaseline," manufactured by the Chesebrough Manufacturing Company, New York, New York. Used in preparation of glass joint lubricant.

Potassium Hydroxide. Pellets, reagent grade, lot No 481635, distributed by Eimer and Amend Company, Inc., New York, New York. Used to absorb carbon dioxide produced during measurements of bacterial oxygen uptake.

Potassium Phosphate, monobasic. Crystalline, analytical grade, lot No 1.548, manufactured by the J. T. Baker Chemical Company, Phillipsburg, New Jersey. Used in preparation of buffer solutions.

Pseudomonas fluorescens. Pure bacterial culture, V. P. I. No 1. Obtained from the Biology Department of Virginia Polytechnic Institute. Used as test organism.

Sodium Acetate. Crystalline, reagent grade, lot No D-313, code No 2191, manufactured by the General Chemical Company, New York, New York. Used in determining the rate of oxygen uptake by Pseudomonas fluorescens on acetate.

Sodium Chloride. Granular, U.S.P., lot No 43213, manufactured by Merck and Company, Inc., Rahway, New Jersey. Used in preparation of manometer fluid.

Sodium Hydroxide. Flake, reagent grade, code 2325, lot No E224, manufactured by the General Chemical Company, New York, New York. Used in preparation of buffer solution.

Tide. Granular, household detergent, manufactured by Procter and Gamble, New York, New York. Used as a cleaning agent for Warburg flasks.

D-Xylose. Powder, C.P., melting point 153 to 154 °C, lot No 484111, distributed by the Fisher Scientific Company, Pittsburgh, Pennsylvania. Used in determining the rate of oxygen uptake by Pseudomonas fluorescens on xylose.

Apparatus

The following apparatus was used in this investigation on the oxygen uptake of Pseudomonas fluorescens:

Autoclave. Steam sterilizer, serial No 94863, manufactured by the American Sterilizer Company, Erie, Pennsylvania. Used for sterilizing growth media.

Balance. Analytical, 0 to 200 gm, chain-o-matic, manufactured by Seederer-Kohlbusch, Inc., Jersey City, New Jersey. Used for analytical weighing in making up test solutions.

Balance. Triple beam, 0 to 610 gm, patent No 1732612, manufactured by the Ohaus Company, Newark, New Jersey. Used for rough weighing in making up media and buffer.

Centrifuge. Chemical, maximum speed 3,000 rpm, 110 v, ac or dc, manufactured by the International Equipment Company, Boston, Massachusetts. Used in harvesting organisms for tests.

Colorimeter. Klett-Summerson photoelectric, No 7093, 115 v, ac, manufactured by the Klett Manufacturing Company, New York, New York. Used for measuring the quantity of bacteria present in test suspensions.

Cooling Coil. A cooling coil was constructed and installed in the Precision Warburg micro-respirometer. The coil was designed to maintain a bath temperature of 15.5 °C (cooling water temperature 14.5 ± 0.5 °C) under summer conditions with air temperatures of 29 ± 1 °C. The coil was constructed of 3/8 inch 18 B.W.G. soft copper tubing and had a rectangular shape 34.8 by 8 inches, with bends of a 2-inch radius. The coil consisted of three loops spaced 7/8 inch apart on centers. Coil entered the back top of the bath with exit and entry tubes spaced 4 inches apart on centers and cleared the back wall by 11/16 inch on centers. Coil cleared the bottom of the tank by 1-1/2 inches on centers on the low side and 1-11/16 inches on centers on the high side. Coil proper cleared the sides of the tank by 7/8 inch on centers. The effective cooling length of the coil was 21.4 feet.

Drying Oven. Laboratory style, 40 to 95 °C, 110 v, 660 w, manufactured by the Will Corporation, Rochester, New York. Used for drying glassware.

Incubator. Model 70, 115 v, 400 w, manufactured by the Electric Hotpack Company, Inc., Fox Chase, Pennsylvania. Used for incubating cultures.

Miscellaneous Glassware. Standard laboratory glassware was used throughout this investigation. Glassware was obtained from the stockroom of the Chemical Engineering Department of Virginia Polytechnic Institute.

pH Meter. Industrial type, Beckman model H-2, 110 v, 50 to 60 cy, ac, manufactured by the National Technical Laboratories, South Pasadena, California. Used to check the pH of buffers used in the investigation.

Refrigerator. Household "Frigidaire," 7.7 cu ft, Pc No 5858351, 10-2-48 - 295M(33), model ML-93, manufactured by the Frigidaire Division of General Motors Corporation, Dayton, Ohio. Used as a cold source for stocking cultures and media.

Stirrer. Varispeed, 110 v, 60 cy, ac, manufactured by the Precision Scientific Company, Chicago, Illinois. Used for preparing and agitating bacterial suspensions.

Timer. "Precision Time-it," 0 to 9999.9 seconds by 0.1 second increments, manufactured by the Precision Scientific Company, Chicago, Illinois. Used for timing all tests made in this investigation.

Warburg Flasks. Catalog No 5-202, manufactured by the American Instrument Company, Silver Spring, Maryland, and catalog No CEW-125, manufactured by E. Machlett and Son, New York, New York. Used for the manometric determination of bacterial oxygen uptake.

Warburg Manometers. Catalog No 5-200, manufactured by the American Instrument Company, Silver Spring, Maryland, and catalog No CEW 155, manufactured by E. Machlett and Son, New York, New York. Used for the manometric determination of bacterial oxygen uptake.

Warburg Respirometer. "Precision," catalog No 66706, serial No C-6, 115 v, 13 amp, 1500 w, single phase, 60 cy, ac, 10 unit including stand for supporting manometers, manufactured by the Precision Scientific Company, Chicago, Illinois. Used for the manometric determination of bacterial oxygen uptake.

Method of Procedure

The method of procedure used in this investigation on the oxygen uptake of Pseudomonas fluorescens was as follows:

Calibration of Warburg Manometers and Flasks. In Warburg technique constant volume measurements are used. It was, therefore, necessary to know the exact volume of the closed system. The volume can be determined quite accurately by means of mercury calibration. The technique used in this investigation was suggested by Burris⁽¹¹⁵⁾.

The flasks and manometers were first thoroughly cleaned and then dried. A permanent reference mark was placed about one centimeter above the ground glass joint on each manometer. One of the flasks was weighed empty and then filled with clean, dry mercury. The flask was then carefully inspected, and all trapped air bubbles were teased out using a capillary pipet. In this connection it was also quite possible to trap air in or around the center well. It was, therefore, advisable to run the pipet around the inside and outside of the well to remove these bubbles. An even better procedure was to fill the well first and remove bubbles while they were visible, after which the flask was completely filled. The removal of air bubbles is of prime importance because they may occupy a considerable volume and thereby cause appreciable error in the final results.

When all bubbles were removed the flask was placed on the dry manometer joint and seated. The mercury was allowed to rise in the capillary of the manometer. If it did not stop exactly on the mark on the manometer, mercury was either removed from or added to the flask with a capillary pipet. When the mercury rose exactly to the mark on the manometer, the flask was removed, the temperature of the mercury was taken, and the flask and mercury were weighed. The weight of the mercury was obtained by subtracting the weight of the empty flask from the weight of the flask plus mercury. The volume of the flask plus the volume of the capillary of the manometer up to the mark was obtained by dividing the weight of mercury by the density of mercury at the temperature of the determination.

The volume of the manometer must also be determined to obtain the total volume of the closed system. For this purpose a rubber stopper with a hole of appropriate size was placed about half way on the ground glass joint of the manometer. When the manometer was inverted the hole in the stopper formed a small well over the joint. The manometer was inverted and a small amount of mercury was poured into the well over the ground glass joint. Care was taken that no air was trapped in the mercury column formed in the manometer. The stopcock on the right leg of the manometer was opened slightly to allow the mercury to drop into contact with

the stopcock. The rubber stopper was then removed and the excess mercury was dumped into a prepared container. The manometer was tipped until the end of the mercury column coincided with the mark above the joint. The other end of the column was in the graduated section of the right leg, and the reading of the terminal point of this column was recorded. The mercury was then dumped into a tared weighing bottle and weighed. Ambient temperature was considered to be the temperature of the mercury at this point. Ambient temperature was the actual temperature of the mercury if the operator had taken care to handle the manometer in such a way that his hands did not touch the glass capillary containing the mercury column.

This procedure was repeated using a shorter column of mercury and tipping the manometer so that the entire column was within the graduated portion of the right leg. The number of millimeters of the manometer occupied by this column was recorded and the mercury again dumped and weighed. From this last operation the volume of the manometer per millimeter of graduation can be calculated. Knowing this value, and knowing the millimeters that the mercury column came into the graduated section when the other end of the column was at the mark above the joint, one can easily calculate the volume of the uncalibrated section of the right leg of the manometer. The total volume of the enclosed system was then the

sum of the flask volume, the volume of the ungraduated section of the right leg, and the volume of the graduated section down to the manometer fluid. The volume of the flask and ungraduated section are constants and the volume of the graduated section can easily be calculated from the known value of the volume per millimeter of graduation.

Preparation of Manometer Fluid. The manometer fluid used in this investigation was the so-called Brodie's solution. According to Umbreit et al. ⁽¹¹²⁾ 23 grams of sodium chloride and 5 grams of oxbile dissolved in water and made up to 500 milliliters of solution will give the desired fluid. This procedure was followed, but the resulting mixture was extremely cloudy. To overcome this cloudiness the mix was filtered through a buchner funnel using "super cell" filter aid. The resulting filtrate was quite clear. The solution was then colored with Evans blue. The dye was added in small amounts until the solution was deep blue. The density of the solution was determined by standard pycnometric methods. The density was found to be 1.0273 grams per milliliter at 26.5 °C. The manometers were filled with this fluid and this same fluid was used throughout the investigation.

Stocking of Cultures. The original culture of Pseudomonas fluorescens was obtained from the Biology Department of Virginia Polytechnic Institute. This culture was streaked onto a nutrient

agar slant and incubated at 30 ± 2 °C for 24 hours. The incubated slants were placed in the refrigerator at 5 °C and left there until needed. Stocks were transferred once a month to insure viability.

Growing of Organisms for Tests. All organisms used in the tests in this investigation came originally from the previously mentioned stocks. A small amount of growth was aseptically transferred from stock to a culture tube containing about 8 milliliters of nutrient broth. This broth inoculum was incubated at 30 ± 2 °C for 24 hours. In the early part of the investigation 1 milliliter of the broth culture was aseptically transferred to 400 milliliters of sterile broth contained in a liter erlenmeyer flask. The 400 milliliter culture was incubated for 20 to 24 hours and then harvested for the tests. The 1 milliliter-in-400 milliliter inoculum grew successfully in all cases, but normally gave a small harvest. For this reason it was decided to use a larger inoculum, and in the latter part of the investigation the 400 milliliters of broth were inoculated with 5 milliliters of a 24-hour broth culture. With the 5-milliliter inoculum the 400-milliliter cultures always gave an adequate harvest at the end of 20 to 24 hours of incubation. One final refinement was made in growing the initial inoculum. The organisms were cultured in 5 milliliters of broth contained in a 50-milliliter erlenmeyer flask rather than a

culture tube. This gave a shallow broth with a volume to exposed surface area ratio of 1:3, which in turn gave highly aerobic conditions favorable to the growth of Pseudomonas fluorescens.

Calibration of Klett Summerson Photoelectric Colorimeter.

The colorimeter was calibrated so that a curve of dry bacterial cell weight in suspension versus colorimeter reading could be plotted. To do this, 400 milliliters of a 24-hour culture of Pseudomonas fluorescens were centrifuged at 2,000 revolutions per minute to settle the cells. The supernatant culture medium was poured from the cells, and the cells were suspended in 50 milliliters of distilled water. The suspended cells were mechanically agitated for 15 minutes and then centrifuged again to settle the cells. The supernatant water was poured from the cells, and the cells were resuspended in 25 milliliters of distilled water. After 15 minutes agitation a 5-milliliter aliquot of the suspension was taken and analyzed in the colorimeter. The colorimeter reading was recorded. Three 5-milliliter aliquots of the original suspension were then pipetted into tared 50-milliliter beakers, and dried for 24 hours at 85 °C. The dried suspension was weighed and then placed in a desiccator over calcium chloride until it reached constant weight. The original suspension was diluted into four more samples of 1/2, 1/4, 1/8, and 1/16 the strength of the original. These samples

were placed in the colorimeter and the colorimeter readings for the dilutions were recorded. The dry weight of the cells contained in these solutions was calculated from the gravimetric analysis of the original suspension. The function of the dry weight of bacteria contained in the suspensions versus the colorimeter readings for the suspensions were plotted to give the desired calibration curve for the colorimeter.

Preparation of Buffer Solution. All bacterial suspensions and all substrate solutions used in the tests were made up in a buffer solution. In order to make tests as uniform as possible in this respect, the buffer for the entire investigation was made up at one time. The buffer used was a 0.05 molar phosphate buffer of pH 6.8. Directions for making up the buffer are given in Lange's Handbook of Chemistry⁽⁴⁵⁾. Ten liters of buffer were made up and the pH was adjusted to 6.8.

Preparation of Bacterial Suspensions. To use the resting cell technique^(34,56,113,118,119) in the tests it was necessary to free the cells from their growth medium and suspend them in a buffer. The 400-milliliter, 20 to 24-hour culture of Pseudomonas fluorescens was first centrifuged to give a primary separation of cells from medium. Centrifugation was performed in a clinical type centrifuge employing 50-milliliter tubes. The speed of centrifugation was 2,000 revolutions per

minute and the time of centrifugation was 30 minutes. The supernatant medium was poured from the settled cells after centrifugation. The medium was discarded and the cells were suspended in 50 milliliters of 0.05 molar phosphate buffer of pH 6.8. The cell suspension was mechanically agitated by means of a laboratory stirrer for a period of 15 minutes. This stirring served the purpose of washing the cells from their attached growth medium. After agitation the buffer suspension was again centrifuged at 2,000 revolutions per minute for a period of 20 minutes. The supernatant buffer was discarded and the cells were resuspended in 25 milliliters of fresh buffer. The suspended cells were again mechanically stirred for a total period of 30 minutes. The 30-minute washing period was found experimentally to give cells with a high respirational activity and a low endogenous rate of oxygen uptake. After 15 minutes of agitation a 5-milliliter sample of the suspension was analyzed in the colorimeter, and the weight of dry bacterial protoplasm contained was determined from the colorimeter calibration curve. On the basis of this information the suspension was volumetrically diluted to give a final suspension of 1.5 milligrams of dry cells per milliliter of suspension. The diluted sample was rechecked on the colorimeter and adjustment made, if necessary. At the end of

agitation period the standardized suspension was either used immediately or stored under refrigeration at 5 °C. Suspensions were never stored more than 5 hours before use.

Preparation of Test Solutions. The test solutions or substrate solutions of D-glucose, sodium acetate, D-xylose, and L-arabinose were made by dissolving the solids in 0.05 molar phosphate buffer of pH 6.8. The glucose and acetate were weighed in sufficient quantities to make 25 milliliters of 0.200 and 1.600 molar solutions. All other concentrations were prepared from the 0.200 or 1.600 molar solutions by volumetric dilution. The xylose and arabinose were weighed in sufficient quantities to make 25 milliliters of 1.600 molar solutions. All other concentrations were prepared from the 1.600 molar solution by volumetric dilution. Solutions were not stocked for more than 8 days in any case.

Preparation of Potassium Hydroxide Solution. A 20-per cent solution of potassium hydroxide was prepared by dissolving 20 grams of the pellets in 80 milliliters of distilled water. The solution was stored in a stoppered bottle and was made up fresh every 60 days. No effort was made to prepare this solution with extreme accuracy since as a carbon dioxide absorbent the alkali was in excess in the tests by at least a factor of 20.

Preparation of Warburg Flasks for Tests. The flasks used in the tests were clean and dry. The rim of the center well or alkali cup was first greased lightly with a 50 per cent lanolin 50 per cent petroleum jelly grease⁽⁵⁷⁾. Following the greasing, 0.20 milliliter of 20 per cent potassium hydroxide solution was pipetted into the center well of the flask. Two milliliters of a suspension of bacterial cells (1.5 milligrams of dry bacterial protoplasm per milliliter) were pipetted into the body of the flask. One-half a milliliter of the test solution desired was pipetted into the side arm of the flask. The vent tube was greased and placed in the side arm mouth of the flask. A one square inch piece of filter paper was folded and placed in the center well in contact with the alkali solution. The manometer joint was greased and the flask was connected to the manometer. The stopcock on the flask side of the manometer was left open to the atmosphere.

General Pretest Preparations. Before tests were started the thermostatic control for the constant temperature bath of the Warburg micro-respirometer was adjusted to the temperature desired for the test. After the bath had come to temperature, the 10 manometer-flask assemblies were attached to the Warburg apparatus by means of the manometer clamps. The manometer-flask

agitator was put into action and flasks were agitated for 15 minutes at a rate of 130 cycles per minute. This agitation period was for the purpose of bringing the flasks and their contents to the temperature of the bath and also of establishing equilibrium between the gas phase and the liquid phase in the flask. At the end of this period, the manometer fluid was adjusted to the 25-centimeter mark on the flask side (right side) of the manometer and the stopcock on the flask side of the manometer was closed to the atmosphere. The height of the fluid on the atmospheric side (left side) of the manometer was read and recorded.

Performance of Tests and Data Taken. At time zero in the tests, the manometers were each removed from the Warburg apparatus, the contents of the side arm were dumped into the body of the flask by tipping the manometers, and the manometers were placed back on the apparatus. The manometer-flask agitator was started. Data were taken at 10-minute intervals in the following manner: the agitator was stopped, the manometer fluid in the right side (flask side) of the manometer was adjusted to the 25-centimeter mark, the reading of the left leg was recorded, and the agitator was started again. The tests were continued for 70 minutes or until the test solution was exhausted (as evidenced by a falling off of rate), whichever

occurred first. When tests were complete, the agitator was stopped, the stopcock on the right side of the manometer was opened to relieve the pressure on the manometer fluid, and the manometers were removed from the apparatus.

Cleaning of Flasks. Upon completion of the tests the flasks were removed from the manometers and the vent tubes were removed from the flasks. All grease was wiped from the flasks with a cotton swab soaked in benzene. The degreased flasks were rinsed with tap water and then boiled for 30 minutes in a Tide solution (about 4 grams of Tide per liter). After boiling, the flasks were rinsed three times in tap water and three times in distilled water, and were then dried in a drying oven at 90 °C. Several check calibrations indicated that no detectable change in flask volumes were caused by this washing and drying procedure.

Data and Results

The data and results obtained in this investigation on the rate of oxygen uptake of Pseudomonas fluorescens are presented in this section. Table III presents the calibration data for the colorimeter and Figure 3 is a plot of these data. Table IV and Table V show the data and results obtained in the calibration of the Warburg flasks and manometers. Table VI presents the data obtained in a study of the effect of concentration on the rate of oxygen uptake of Pseudomonas fluorescens, and Figure 4 is a graphical representation of these data. Table VII presents the data for the effect of temperature on the rate of oxygen uptake of Pseudomonas fluorescens respiring on D-glucose, sodium acetate, D-xylose and L-arabinose. Figure 5 is a graphical representation of the data of Table VII.

Subtraction of Endogenous Rates. When the data obtained in the experimental tests were analyzed it was found that for any given conditions of temperature and substrate, total uptakes and endogenous uptakes were not constant from test to test. However, when endogenous uptakes were subtracted from corresponding total uptakes, the value obtained was constant and reproducible for all tests made under the conditions chosen. For this reason,

all tables and figures showing the rate of oxygen uptake as a function of substrate concentration and environmental temperature, endogenous rates have been subtracted from total rates to give rate of uptake caused by the oxidation of substrate. According to Orcutt⁽¹²⁵⁾ this method of presentation has been questioned by many workers in the field, since various substrates may suppress or enhance the endogenous rate to various degrees. However, there is precedence in the modern literature for this method of presentation^(41,123,126,127). Also, it is the personal opinion of the investigator that in this case, the question of whether or not to subtract endogenous rates is somewhat academic for the following reasons: first, the general shapes of the curves obtained will be the same whether or not endogenous rates are subtracted. Second, when tests were being made, all substrates were tested at the same time and with only one endogenous rate being taken, so if subtraction of endogenous rate is an error, it is compensated for by the design of the experiment.

TABLE III

Colorimeter Readings and Weights of Dry Bacterial
Protoplasm for Distilled Water Suspensions
of Pseudomonas Fluorescens

<u>Colorimeter</u> <u>Readings</u>	<u>Dry Bacterial</u> <u>Weight</u>
<u>Scale</u>	<u>mg per ml</u>
545	2.00
330	1.00
195	0.50
106	0.25
54	0.12

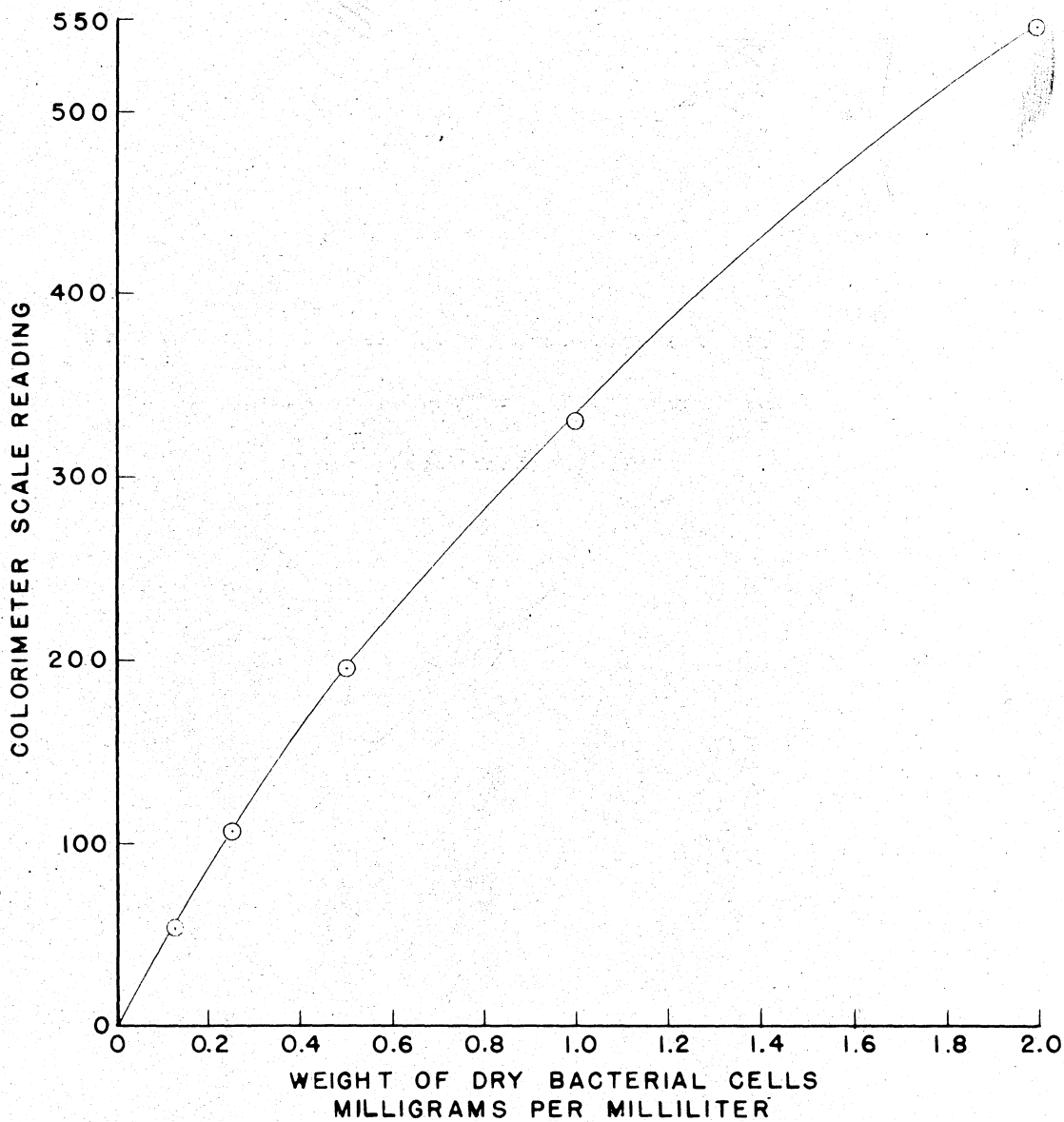


FIGURE 3. CALIBRATION CURVE FOR KLETT-SUMMERSON PHOTOELECTRIC COLORIMETER FOR CONVERSION OF COLORIMETER SCALE READINGS TO DRY WEIGHT OF PSEUDOMONAS FLUORESCENS PER MILLILITER OF AQUEOUS SUSPENSION

TABLE IV

Weights of Mercury Contained by Warburg Flasks
and the Calculated Volume of the Flask

Companion Manometer	Flask	Test No.									Average Calculated Volume ml
		1			2			3			
		Weight of Mercury Contained g	Tempera- ture °C	Calculated Volume ml	Weight of Mercury Contained g	Tempera- ture °C	Calculated Volume ml	Weight of Mercury Contained g	Tempera- ture °C	Calculated Volume ml	
No	No										
1	1	178.4827	29.0	13.19742	178.4804	29.0	13.19725	178.4774	29.0	13.19702	13.19723
2	2	181.0565	29.0	13.38773	181.0610	29.0	13.38807	181.0615	29.0	13.38810	13.38797
3	3	185.1453	29.5	13.69127	185.1452	29.5	13.69126	185.1425	29.5	13.69106	13.69120
4	4	181.2846	29.5	13.40578	181.2886	29.5	13.40607	181.2834	29.5	13.40569	13.40585
5	5	174.1876	31.0	12.88450	174.1826	31.0	12.88413	174.1858	31.0	12.88437	12.88433
6	6	180.7394	31.0	13.36913	180.7336	31.0	13.36870	180.7330	31.0	13.36866	13.36883
7	7	230.4368	27.0	17.03280	230.4362	27.0	17.03276	-	-	-	17.03278
8	8	227.6632	28.0	16.83089	227.6587	28.0	16.83055	-	-	-	16.83072
9	9	246.0005	27.0	18.18320	245.9981	27.0	18.18302	-	-	-	18.18311
10	10	244.4029	26.0	18.06191	244.4025	26.0	18.06188	-	-	-	18.06190
7	17	238.7747	26.0	17.64597	238.7713	26.0	17.64572	-	-	-	17.64584
8	18	239.7955	26.0	17.72142	239.7960	26.0	17.72145	-	-	-	17.72145
9	19	254.7714	25.0	18.82475	254.7693	25.0	18.82460	-	-	-	18.82468
10	20	232.3253	28.0	17.17555	232.3213	28.0	17.17526	-	-	-	17.17540
R ^a	R ^a	188.3516	31.0	13.93220	188.3576	31.0	13.93264	188.3565	31.0	13.93256	13.93247

^a Spare manometer and flask reserved against breakage.

TABLE V

Weights of Mercury Contained by Manometers During Calibration,
and the Calculated Manometer Volumes

Manometer	Average Weight ^b of Mercury Contained in Ungraduated Section of Manometer	Temperature of Mercury	Average Volume of Ungraduated Section of Manometer	Average Weight ^a of Mercury Contained in one cm of Graduated Section of Manometer	Temperature of Mercury	Average Volume of one cm of Graduated Section of Manometer
No	gm	°C	ml	gm	°C	ml
1	6.9876	32	0.51696	0.2416	32	0.01787
2	7.7838	33	0.57596	0.2736	33	0.02024
3	8.1679	33	0.60622	0.3048	33	0.02255
4	7.5053	31	0.55515	0.2917	31	0.02158
5	7.4824	32	0.55356	0.2825	32	0.02090
6	7.3103	31	0.54073	0.2379	31	0.01760
7	4.8871	29	0.36136	0.1899	29	0.01404
8	5.0664	29	0.37440	0.2006	29	0.01483
9	4.8828	29	0.36105	0.1831	29	0.01354
10	5.4734	26	0.40184	0.2011	26	0.01486
R ^c	8.7683	30	0.64845	0.2687	30	0.01987

^a Average of three determinations.

^b Average of two determinations.

^c Spare manometer reserved against breakage.

TABLE VI

Effect of Concentration of Substrate on the Rate of Oxygen Uptake of 3.0 Milligrams
of Pseudomonas Fluorescens Suspended in 2.5 Milliliters of 0.05 Molar Phosphate
Buffer of pH 6.8 at a Reaction Temperature of 25 °C

Investigation No	Substrate	Substrate Concentration Millimoles per Test	Number of Tests Made No	Average Rate of Oxygen Uptake $\mu\text{l per Hr}^{a,b}$	Average Deviation $\mu\text{l per Hr}^c$	Maximum Positive Deviation $\mu\text{l per Hr}$	Maximum Negative Deviation $\mu\text{l per Hr}$
1	Glucose	0.8000	2	125	2	2	2
2	Glucose	0.4000	2	128	1	1	1
3	Glucose	0.2000	2	135	3	3	3
4	Glucose	0.1000	6	135	3	5	6
5	Glucose	0.0500	2	131	2	2	2
6	Glucose	0.0250	4	103	2	3	3
7	Glucose	0.0125	2	74	10	10	10
8	Glucose	0.0062	6	78	6	8	11
9	Glucose	0.0031	2	51	4	4	4
10	Glucose	0.0016	4	36	6	8	13
11	Glucose	0.0008	2	16	2	2	2
12	Sodium Acetate	0.8000	4	-17	2	3	2
13	Sodium Acetate	0.4000	2	27	1	1	1
14	Sodium Acetate	0.2000	4	73	2	3	5
15	Sodium Acetate	0.1000	4	115	1	1	2
16	Sodium Acetate	0.0500	16	117	4	20	10
17	Sodium Acetate	0.0250	4	115	6	6	9
18	Sodium Acetate	0.0125	4	108	7	9	8
19	Sodium Acetate	0.0062	6	93	4	5	7
20	Sodium Acetate	0.0031	2	82	2	2	2
21	Sodium Acetate	0.0016	2	79	6	6	6
22	Sodium Acetate	0.0008	4	75	9	10	16
23	Xylose	0.8000	2	143	1	1	1
24	Xylose	0.6000	2	143	7	7	7
25	Xylose	0.4000	6	126	6	10	5
26	Xylose	0.2000	2	111	1	1	1
27	Xylose	0.1000	2	84	1	1	1
28	Arabinose	0.8000	2	93	1	1	1
29	Arabinose	0.4000	6	60	3	5	6
30	Arabinose	0.2000	2	43	1	1	1
31	Arabinose	0.1000	2	24	1	1	1
32	Sodium Acetate Sodium Chloride	0.0500 0.7500	2	96	1	1	1

^a Endogenous rates have been subtracted to give these values.

^b Arithmetic mean of tests.

^c Arithmetic average deviation of tests from mean.

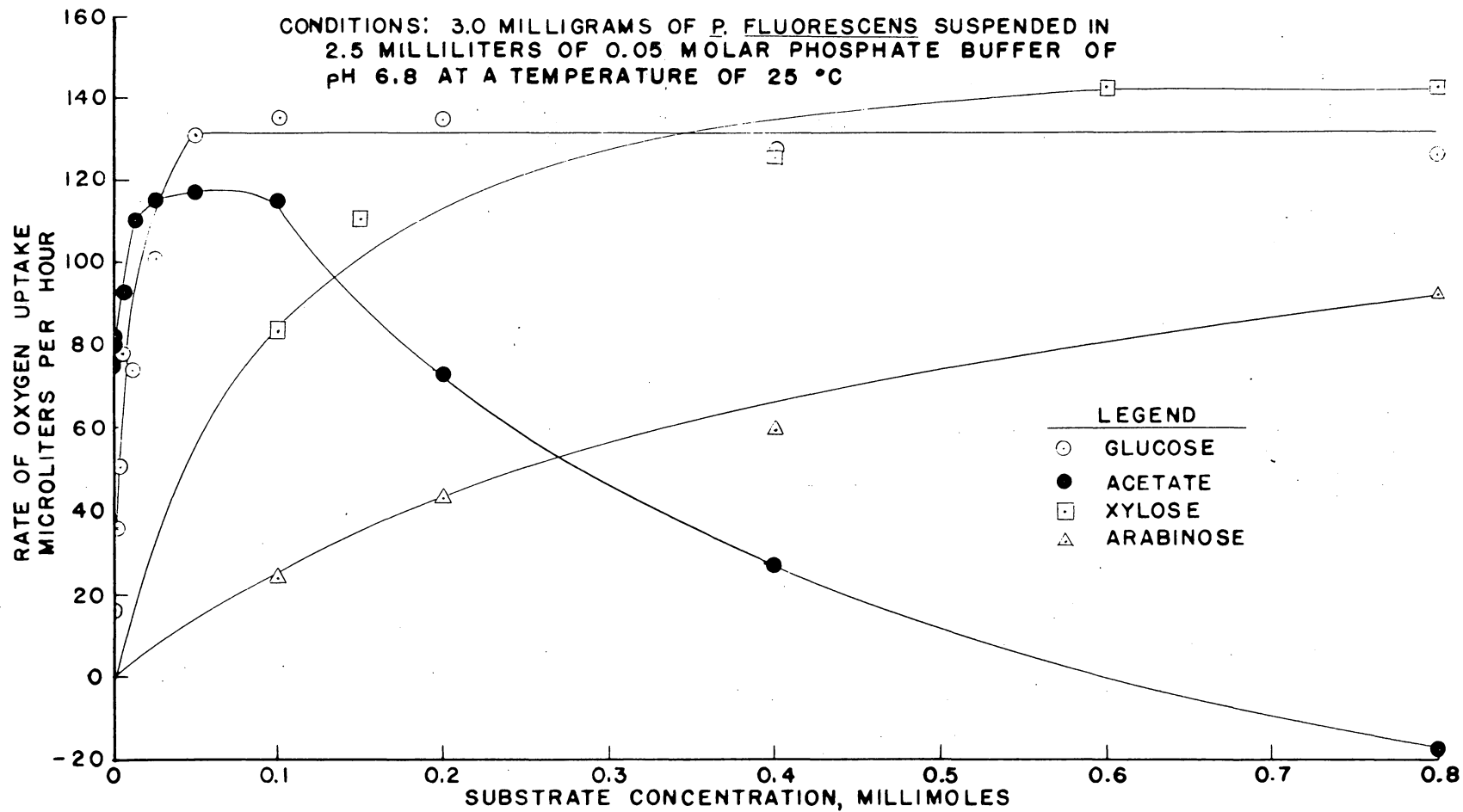


FIGURE 4. EFFECT OF CONCENTRATION OF SUBSTRATE ON THE RATE OF
OXYGEN UPTAKE BY *PSEUDOMONAS FLUORESCENS*

TABLE VII

Effect of Temperature on the Rate of Oxygen Uptake of 3.0 Milligrams
of Pseudomonas Fluorescens Suspended in 2.5 Milliliters
of 0.05 Molar Phosphate Buffer of pH 6.8

Investigation	Substrate	Substrate Concentration	Temperature	Number of Tests Made	Average Rate of Oxygen Uptake	Average Deviation	Maximum Positive Deviation	Maximum Negative Deviation
No		Millimoles per Test	°C	No	$\mu\text{l per Hr}^{\text{a,b}}$	$\mu\text{l per Hr}^{\text{c}}$	$\mu\text{l per Hr}$	$\mu\text{l per Hr}$
1	Glucose	0.1000	15.0	4	44	2	2	3
2	Glucose	0.1000	17.5	4	86	6	7	7
3	Glucose	0.1000	20.0	2	123	2	2	2
4	Glucose	0.1000	25.0	6	135	3	5	6
5	Glucose	0.1000	30.0	7	183	12	17	16
6	Glucose	0.1000	35.0	8	245	31	29	41
7	Glucose	0.1000	37.0	7	286	5	8	12
8	Sodium Acetate	0.0500	15.0	4	45	7	11	7
9	Sodium Acetate	0.0500	17.5	4	94	6	6	11
10	Sodium Acetate	0.0500	20.0	3	106	5	6	9
11	Sodium Acetate	0.0500	25.0	16	117	4	20	10
12	Sodium Acetate	0.0500	30.0	5	142	8	16	19
13	Sodium Acetate	0.0500	35.0	6	213	18	25	23
14	Sodium Acetate	0.0500	37.0	6	230	6	10	16
15	Xylose	0.4000	15.0	4	40	2	4	4
16	Xylose	0.4000	17.5	4	88	5	6	5
17	Xylose	0.4000	20.0	6	103	5	10	7
18	Xylose	0.4000	25.0	6	120	6	10	5
19	Xylose	0.4000	30.0	4	140	4	9	5
20	Xylose	0.4000	35.0	4	161	7	10	7
21	Xylose	0.4000	37.0	6	194	8	16	6
22	Arabinose	0.4000	15.0	4	32	1	1	2
23	Arabinose	0.4000	17.5	2	58	4	5	4
24	Arabinose	0.4000	20.0	2	60	1	1	1
25	Arabinose	0.4000	25.0	6	60	3	5	6
26	Arabinose	0.4000	30.0	4	63	7	8	10
27	Arabinose	0.4000	35.0	4	74	4	6	7
28	Arabinose	0.4000	37.0	4	80	5	10	5

^a Endogenous rates have been subtracted to give these values.

^b Arithmetic mean of tests.

^c Arithmetic average deviation of tests from mean.

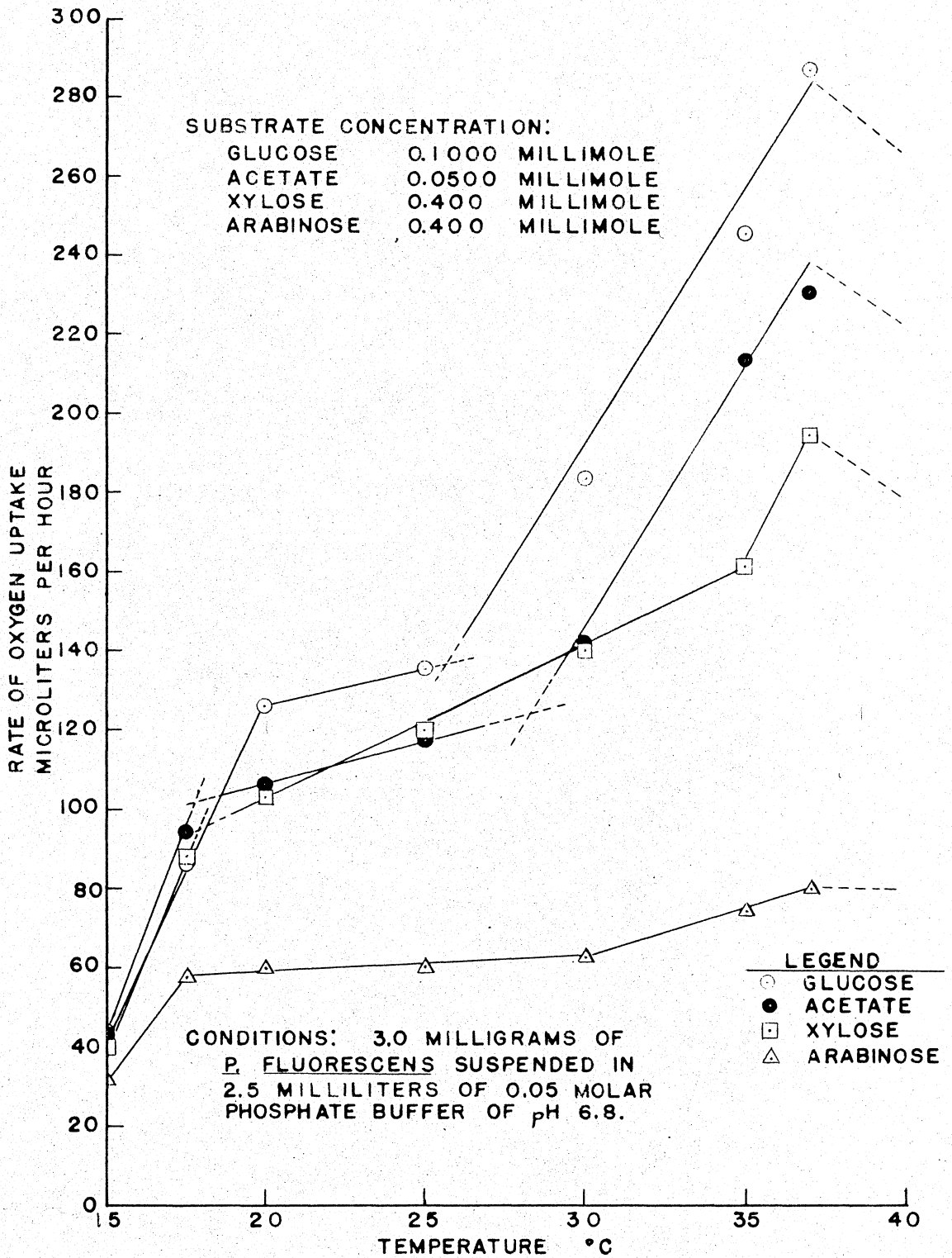


FIGURE 5. EFFECT OF TEMPERATURE ON THE RATE OF OXYGEN UPTAKE BY *PSEUDOMONAS FLUORESCENS*

Studies on Respiration of Bacillus Subtilis. One requirement for the Doctor's Degree in Chemical Engineering is that the candidate shall supervise a research project of some student who is a candidate for a lesser degree. The project shall be closely related to the doctorate research. The author of this thesis supervised a research project performed by S. E. Ketner⁽⁴²⁾. The purpose of Ketner's thesis was to obtain fundamental information concerning stream pollution by studying the effect of substrate concentration and environmental temperature on the rate of oxygen utilization by a typical water bacterium, Bacillus subtilis. The substrates oxidized were glucose and acetate, representative materials of wood pulp waste. Table VIII presents the data obtained in a study of the effect of concentration of D-glucose and sodium acetate on the rate of oxygen uptake by Bacillus subtilis. Figure 6 is a plot of the data given in Table VIII. Table IX shows the data obtained in the investigation of the effect of temperature on the rate of oxygen uptake by Bacillus subtilis respiring on D-glucose and sodium acetate. Figure 7 is a graphical representation of the data given in Table IX. These data and results are presented herewith as evidence of fulfillment of the departmental requirement concerning research supervision.

TABLE VIII

Total Oxygen Uptake of 2.8 Milligrams of Bacillus
Subtilis Respiring on Varying Amounts of Glucose
and Sodium Acetate in a 2.5 Milliliter
Volume at 25 °C

Substrate	Substrate Conc. Millimoles	Rate of O ₂ Uptake μl per hr
Glucose	0.1000	70.5
Glucose	0.1000	69.0
Glucose	0.0500	71.1
Glucose	0.0500	72.7
Glucose	0.0250	83.4
Glucose	0.0250	79.0
Glucose	0.0125	74.8
Glucose	0.0125	69.9
Sodium Acetate	0.1000	20.9
Sodium Acetate	0.1000	17.3
Sodium Acetate	0.0500	24.8
Sodium Acetate	0.0500	32.4
Sodium Acetate	0.0250	26.8
Sodium Acetate	0.0250	23.4
Sodium Acetate	0.0125	27.2
Sodium Acetate	0.0125	29.8

Ketner, S. E.: Effect of Temperature on Oxygen Uptake of Bacillus Subtilis on Glucose and Sodium Acetate, p. 30. Unpublished B. Sc. Thesis, Library, Va. Poly. Inst., Blacksburg, Va., (1950).

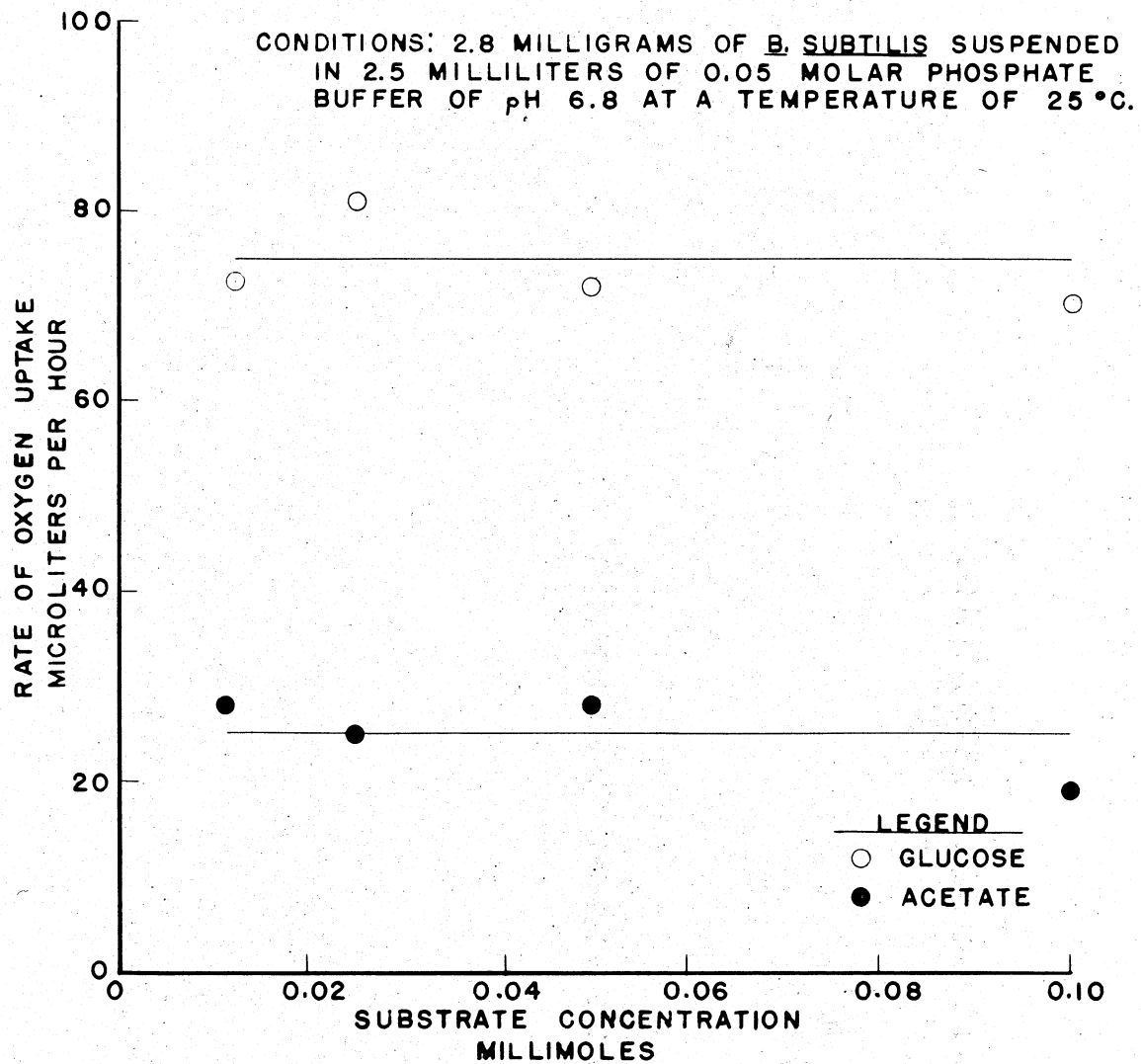


FIGURE 6. EFFECT OF CONCENTRATION OF SUBSTRATE ON THE RATE OF OXYGEN UPTAKE BY BACILLUS SUBTILIS

KETNER, S. E.: EFFECT OF TEMPERATURE ON OXYGEN UPTAKE BACILLUS SUBTILIS ON GLUCOSE AND SODIUM ACETATE, P. 30. UNPUBLISHED B. SC. THESIS, LIBRARY, VA. POLY. INST., BLACKSBURG, VA. (1950).

TABLE IX

The Oxygen Uptake of 2.8 Milligrams of Bacillus Subtilis
on Glucose and Sodium Acetate Substrates
at 20, 21, 22.5, 25 and 30 °C

Substrate	Substrate Conc. Millimoles	Temperature °C	Rate of O ₂ Uptake ^a μl per hr
Glucose	0.0500	20.0	23.0
Glucose	0.0500	21.0	40.2
Glucose	0.0500	22.5	57.0
Glucose	0.0500	25.0	66.2
Glucose	0.0500	30.0	78.6
Sodium Acetate	0.0500	20.0	4.8
Sodium Acetate	0.0500	21.0	23.5
Sodium Acetate	0.0500	22.5	33.3
Sodium Acetate	0.0500	25.0	41.6
Sodium Acetate	0.0500	30.0	36.0

a - The endogenous uptake is subtracted from the total uptake to give these values.

Ketner, S. E.: Effect of Temperature on Oxygen Uptake of Bacillus Subtilis on Glucose and Sodium Acetate, p. 31. Unpublished B. Sc. Thesis, Library, Va. Poly. Inst., Blacksburg, Va., (1950).

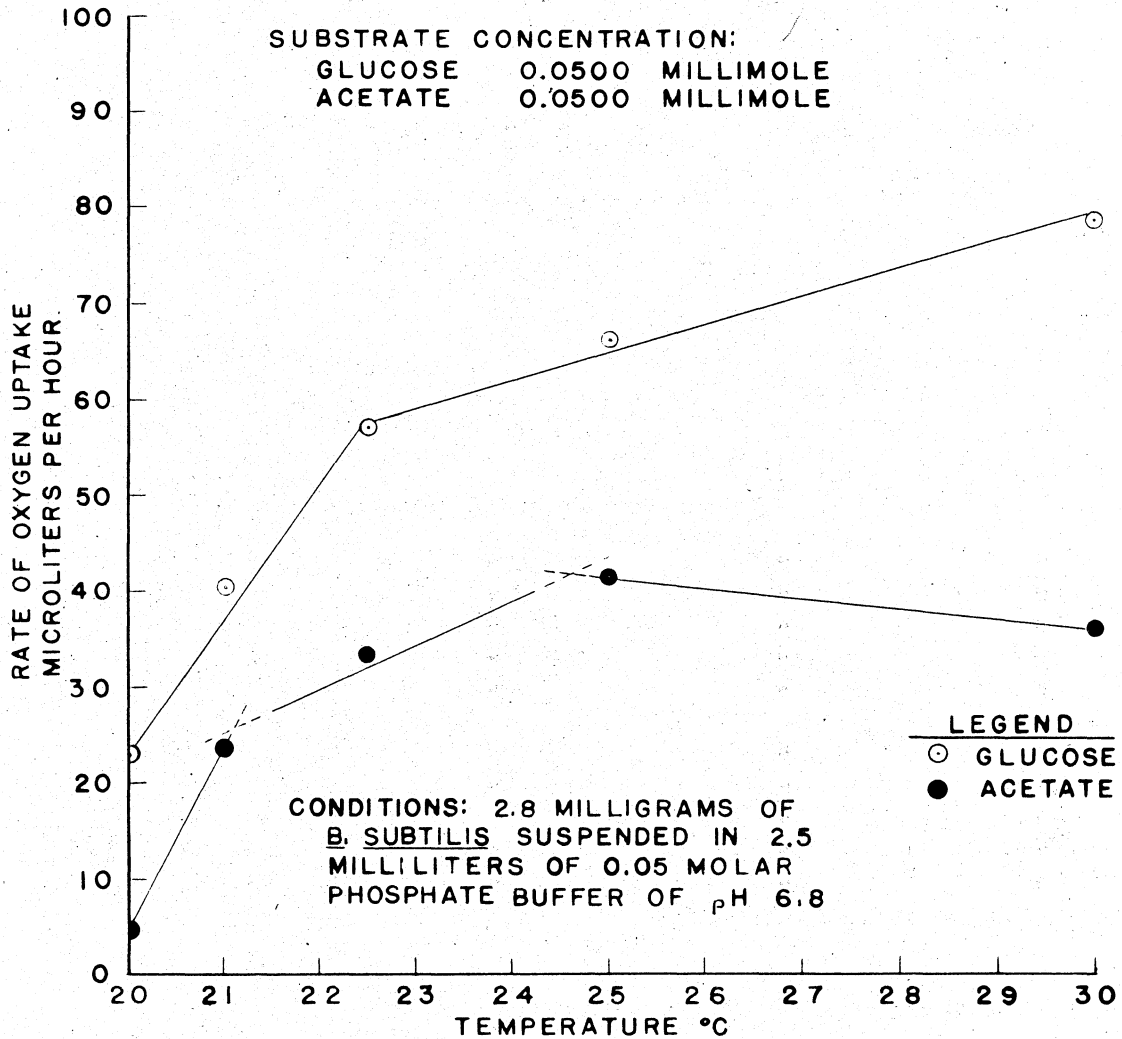


FIGURE 7. EFFECT OF TEMPERATURE ON THE RATE OF OXYGEN UPTAKE BY BACILLUS SUBTILIS

KETNER, S. E.: EFFECT OF TEMPERATURE ON OXYGEN UPTAKE OF BACILLUS SUBTILIS ON GLUCOSE AND SODIUM ACETATE, P. 31. UNPUBLISHED B. SC. THESIS, LIBRARY, VA. POLY. INST. (1950).

Sample Calculations

The calculations involved in this investigation were of only two types: the determination of flask constants, and conversion of manometer readings to volumes of oxygen consumed.

Flask constants were determined by the following equation⁽¹¹²⁾:

$$k = \frac{V_g \frac{273}{T} + V_f \alpha}{P_o}$$

where:

k = the flask constant, microliters of gas
exchanged per millimeter of manometer fluid

V_g = volume of gas in vessel, microliters

273 = standard temperature, degrees Kelvin

T = temperature of bath, degrees Kelvin

V_f = volume of liquid in vessel, microliters

α = solubility of gas involved in the liquid
involved at one atmosphere pressure and
temperature T, microliters of gas per
microliter of liquid

P_o = standard pressure expressed in terms of
manometer fluid, millimeters.

For flask number 2 at 25 °C:

$$k = \frac{12715 \times \frac{273}{298} + 2700 \times 0.02831}{10026} = 1.0217$$

The microliters of gas exchanged was calculated from the observed manometer reading by the following equation⁽¹¹²⁾:

$$x = k h$$

where:

x = volume of gas exchanged, microliters

k = flask constant, microliters of gas per
millimeter of manometer fluid

h = observed manometer reading, millimeters.

IV. DISCUSSION OF RESULTS

The results obtained in this investigation of the effect of substrate concentration and environmental temperature on the rate of oxygen uptake by Pseudomonas fluorescens will be analyzed in this section under the general headings of discussion, recommendations and limitations.

Discussion

The results obtained in this investigation will be discussed and criticized in this portion of the thesis.

Physical and Chemical Techniques. The experimental techniques used in any investigation are, of course, potential sources of errors and should be considered in any discussion of the results obtained. The principal physical and chemical techniques used in the investigation were: centrifugation of cellular suspensions, nephelometric analysis of the cellular suspensions, preparation of substrate solutions, pipetting of substrate solutions and cellular suspensions, and the Warburg techniques. These techniques will be discussed in relation to the possible errors involved, and to their effect on the final results.

Centrifugation of Cellular Suspensions. The cells were settled from their liquid suspensions by means of centrifugation. An ordinary clinical centrifuge was used, with the rate of rotation of approximately 2,000 revolutions per minute, and the time of centrifugation of up to one-half hour. The rate of centrifugation probably varied since line voltage may vary as much as 10 volts. The rate of change in rotational speed would not affect the final results in any manner, except in a variation of the number of cells settled per unit time. However, the temperature of the mixture being centrifuged rose, because of air friction, to 4 5 °C during the one-half hour period. A temperature of 40 °C is not normally fatal to bacteria under natural conditions, but when the cells are packed tightly in the bottom of a centrifuge tube, it is quite possible that the high temperature may cause damage and lower the activity of the organisms. If the cells have been damaged, the results obtained would show rates of oxygen uptake below that of an equal quantity of cells under normal conditions. The potentially lower activity of the cells should be remembered in any attempt to apply the results obtained in the investigation to the uptake expected under natural conditions.

Nephelometric Analysis of Cellular Suspensions. The concentration of suspended cells in milligrams per milliliter was determined nephelometrically. Analyses were done in a Klett-Summerson photoelectric colorimeter. Unfortunately, the suspension used (1.5 milligrams of dry bacterial protoplasm per milliliter) had a colorimeter reading of 454, which is in the upper portion of the scale. The sensitivity of the instrument in this range is such that an error of ± 5 per cent in concentration is within experimental error. However, since tests were made in duplicate, and usually in quadruplicate or higher, it is felt that the error would be largely compensated and that nephelometric analysis was not a source of an error as great as 5 per cent.

Preparation of Substrate Solutions. Standard substrate solutions were prepared by weighing the desired quantity of substrate on an analytical balance and dissolving it in a measured quantity of 0.05 molar phosphate buffer of pH 6.8. Standard solutions were 1.6000 molar and 0.2000 molar for glucose and sodium acetate, and 1.600 molar for xylose and arabinose. Other concentrations were prepared from the standards by volumetric dilution. The probable error in these techniques would be less than 0.1 per cent. The one other possible error in substrate solutions would be in the

purity of the pentose sugars. The sugars used were graded as C.P. and were furnished by a reputable distributor. However, pentoses are in general rare chemicals and their purity is open to some question. Therefore, although weighings were made to five-place accuracy, only three-place accuracy is claimed for the pentose substrate solutions. It is believed that the concentration of the solutions is accurate within this range.

Pipetting of Substrate Solutions and Cellular

Suspensions. Substrate solutions and cellular suspensions were both diluted and transferred by means of graduated serological type pipets. The serological pipet is admittedly not as accurate as the narrow tipped mohr type pipet, but is a much better pipet for handling suspended materials. The pipets used were top grade calibrated pipets and the maximum possible error incurred in pipetting would not exceed 0.5 per cent for any one operation.

Warburg Techniques. The errors involved in the Warburg techniques would occur in pipetting of materials into the flasks, variations of temperature of the bath during tests, and in the reading of the manometers. The pipetting errors have already been discussed. Bath temperature was never observed to vary sufficiently to give any readable change

on a 0.1 °C division thermometer. The error in reading the manometers is definitely appreciable. The smallest division on the manometers is one millimeter and the nearest estimate that can be made is ± 0.25 millimeter. In this investigation the average reading was about 15 centimeters, the minimum reading about 3 centimeters and the maximum about 25 centimeters. Therefore, the possible manometer errors were an average of 1.7 per cent, a maximum of 8.3 per cent and a minimum of 1.0 per cent.

Bacteriological Techniques. In any investigation in which biological material is handled, standard techniques must be set up and used. The techniques frequently place the living material under highly unnatural conditions. In this investigation Pseudomonas fluorescens was handled in pure culture and data were taken by means of resting cell techniques. All growth conditions were standardized both as to growth medium and temperature. The use of pure culture techniques and resting cell techniques will be discussed and criticized, and the possibility of adaptation, training and mutation of cells during culture and the effect of temperature during cell culture will be discussed in the following paragraphs:

Pure Culture Techniques. Pure culture techniques involve the handling of a single strain of species of bacteria, and subsequent growth of this species in sterile medium in the absence of all foreign species. Pure culture techniques make possible the study of physical and chemical properties and activities of the organisms involved. For example, in this investigation, pure cultures of Pseudomonas fluorescens were used and the oxygen uptake of these organisms was determined on glucose, xylose, arabinose and acetate. Since pure culture were used, it was definitely known that the oxygen utilization obtained were caused by Pseudomonas fluorescens and no other organism. However, the principal criticism directed at pure culture work is the highly unnatural environment. In nature all living forms must co-exist with many other organisms. The coexistence of organisms means that a very lively competition exists under natural conditions which is totally lacking in pure culture. Furthermore, in natural mixed cultures all symbiotic relationships are possible. It is therefore obvious that results obtained in pure culture are open to considerable question as far as representing results that would be obtained under natural conditions. In this investigation no data were taken involving the oxygen uptake of mixed cultures

containing Pseudomonas fluorescens, so it is impossible to make any quantitative statement as to the effect of the presence of other organisms.

Resting Cell Techniques. In this investigation the respirational oxygen uptake of 3.0 milligrams of Pseudomonas fluorescens on glucose, xylose, arabinose, and acetate was determined by means of resting cell techniques (34,56,113, 118,119). The resting cell technique involves suspension of the bacterial cells in a medium which is so constituted as to allow respiration but not growth or reproduction. This is a very artificial situation, and the results obtained are open to a great deal of question so far as representing results obtained under natural conditions. In the case of measurement of oxygen uptake, the oxygen is utilized for the production of cellular energy. It is obvious that under normal conditions energy is required by the cells for growth and multiplication. In the resting cell technique the organisms would have no energy requirement for growth, so would, as a consequence, have a lower oxygen demand per cell than in the proliferating state. In the case of Serratia marcescens, McLean and Fisher⁽⁴⁸⁾ indicate that the oxygen uptake for growth has a definite percentage of the total oxygen uptake. However, these investigators also noted that the percentage

oxygen uptake for growth varied with the nitrogen source, carbon source, and temperature. In view of these results, it is obvious that extrapolation of resting cell results to cover proliferating cell conditions is possible if the data are available. In this investigation no data were taken with proliferating cells, so percentage uptake for growth is not known for Pseudomonas fluorescens. The resting cell technique does have the great advantage of giving a constant cellular concentration during a test. A constant cellular concentration will give reproducible results in any tests on cellular activities. Proliferating cell studies are much harder to interpret than resting cell studies because cell concentration is constantly changing and the cells are changing in activity as they progress through the various growth phases. As a matter of fact, resting cell observations are frequently made in conjunction with proliferating cell studies to aid in interpretation of results.

The Adaptation and Training of Cells During Culture.

Considerable experimental work has shown that bacteria are in possession of two types of respirational enzymes⁽³²⁾: constitutive enzymes which are always present regardless of substrate, and adaptive enzymes, which

appear only in response to the presence of their particular substrates. To give an example, suppose that for a definite species of bacteria the enzymes responsible for the oxidation of glucose are constitutive while the enzymes responsible for the oxidation of galactose are adaptive. If the afore-mentioned organisms were placed in contact with glucose, the constitutive enzymes would immediately begin to oxidize the glucose. However, if the same organisms were placed in contact with galactose while in an actively growing state, the initial rate of oxidation of the hexose would be either zero or extremely low, and then as the adaptive enzymes were formed, the rate would approach a maximum value within a period of a few generations. A second phenomenon similar to adaptation is known as training. However, to train an organism to oxidize a substrate requires several days of subculturing in the presence of the particular substrate, rather than the few generations required for adaptation. While these two phenomena do resemble each other, it is believed that they are not physiologically related. Adaptation is thought to be caused by the cellular synthesis of the required enzyme in the presence of an unnatural substrate. Training

is believed to be caused by changes in cell wall permeability, i. e. the cell wall is not initially permeable to the substrate in question, and slowly becomes permeable with contact. Another theory postulates that training is actually the appearance and growth of a spontaneous mutant.

In this investigation, Pseudomonas fluorescens was grown on nutrient broth in the complete absence of the test substrates (D-glucose, D-xylose, L-arabinose and acetate). The tests were made using the resting cell technique, so the cells were not reproducing when in contact with the test substrates. Under these conditions it would be impossible for the organisms to exhibit either adaptation or training toward the substrates in question, a fact which should be remembered in interpreting results. If Pseudomonas fluorescens is capable of adaptation to any of the substrates tested, the adapted cells would have a higher rate of oxygen uptake than the unadapted cells used in these tests.

Spontaneous Mutation During Culture. It is an established fact that many species of bacteria are capable of spontaneous mutation, and at least some varieties of Pseudomonas fluorescens are known to produce certain mutants⁽²⁸⁾. The production of a mutant strain of organism

would, in all probability, affect the reproducibility of results in an investigation concerning oxygen uptake on various substrates. In this investigation on the oxygen uptake of Pseudomonas fluorescens the possibility of mutation was taken into account and frequent back-checks of results were made. Appreciable deviations were not noted on any of the back-checks, even for a time elapse of as much as four months between determinations. It can therefore be assumed that either mutations did not occur, or if mutations did occur, the mutant had the same characteristics, in regard to the variables tested, as the parent culture.

Effect of Environmental Temperature During Growth of Test Organisms. During the experimental investigation the incubator was accidentally disconnected from the electric power source. Under these conditions, incubation temperature was lowered from 30 ± 2 °C to ambient temperature which would have been approximately 25 °C. The error was not noticed immediately and two tests were made studying the effect of glucose concentration on the rate of oxygen uptake. The yield of cells at harvest was decreased by approximately 50 per cent in both cases, and the rate of oxygen uptake per milligram of dry bacterial protoplasm

was decreased by about 20 per cent. This effect was not studied further since it was not included in the plan of investigation. However, the incubator used did have a control lag of ± 2 °C, so this same effect might have been the cause of some of the variance observed in the experimental results.

Effect of Glucose Concentration on the Rate of Oxygen Uptake by Pseudomonas Fluorescens. For 3.0 milligrams of Pseudomonas fluorescens suspended in 2.5 milliliters of 0.05 molar phosphate buffer of pH 6.8 and at a temperature of 25 °C, the rate of oxygen uptake increases with glucose concentration between the values of 0.0008 and 0.0500 millimole glucose per test. Between substrate concentrations of 0.0500 and 0.8000 millimole per test the rate of oxygen uptake remained constant and independent of substrate concentration. (See Table VI and Figure 4.) To explain the effect of substrate concentration on the rate of oxygen uptake, it must be remembered that the cellular concentration, and therefore the enzyme concentration, was constant for all tests. According to one of the theories of enzyme kinetics⁽¹⁰³⁾, enzymes react with their substrate to form an intermediate compound, which in turn breaks down into products such as carbon dioxide, water, or organic acids, plus the original enzyme. It is further

postulated that the rate of reaction depends upon the concentration of the enzyme-substrate complex. Therefore, when the concentration of the substrate is sufficiently high to saturate all the enzyme present, the concentration of the enzyme-substrate complex would remain constant, and further increases in substrate concentration would not bring about a further increase in reaction rate. On the other hand, if the substrate concentration is not high enough to saturate the enzyme, the concentration of the enzyme-substrate complex, and therefore the reaction rate will be proportional to the substrate concentration. The theory just outlined explains the data and results obtained for glucose, and it seems evident that the saturation concentration of glucose for 3.0 milligrams of Pseudomonas fluorescens (stated as dry bacterial protoplasm) is approximately 0.0500 millimole.

Effect of D-Xylose Concentration on the Rate of Oxygen Uptake of Pseudomonas Fluorescens. For 3.0 milligrams of Pseudomonas fluorescens suspended in 2.5 milliliters of 0.05 molar phosphate buffer at a temperature of 25 °C, the rate of oxygen uptake increases with xylose concentration from 0.100 to 0.600 millimole xylose per test. Between substrate concentrations of 0.600 and 0.800 millimole per test the rate of

oxygen uptake remained constant and independent of concentration. (See Table VI and Figure 4.) In the case of xylose, the saturation concentration is approximately 0.600 millimole or at least a factor of 10 greater than the saturation concentration for glucose. One explanation is that a different series of bacterial enzymes is involved in the oxidation of xylose than the enzymes involved in glucose oxidation. It is also interesting to note that while the saturation concentration for xylose is greater than the saturation concentration for glucose, the rate of oxygen uptake at saturation for xylose is greater than the rate for glucose by a factor of 1.09 ± 0.03 . This difference is small but significant, and might be taken as further indication that different enzymes are involved in the oxidation of glucose and xylose. However, one should be cautious in making an assumption of different enzymes for these two sugars, for the sugars are very similar stereochemically. If the potential aldehyde group is called carbon 1, and the carbons are numbered consecutively down the chain, then D-glucose and D-xylose have the same configuration for the first 4 carbon atoms. It is therefore possible that enzymes utilized in glucose oxidation could attack xylose, and under these conditions it is likely that the saturation concentration would be higher for xylose,

the unnatural substrate, than for glucose, the natural substrate. It is doubtful, though, that an unnatural substrate would be oxidized at a higher rate than the natural substrate.

Effect of L-Arabinose Concentration on the Rate of Oxygen Uptake of Pseudomonas Fluorescens. For 3.0 milligrams of Pseudomonas fluorescens suspended in 2.5 milliliters of 0.05 molar phosphate buffer of pH 6.8 and at a temperature of 25 °C the rate of oxygen uptake increases with arabinose concentration between the values of 0.100 and 0.800 millimole per test. (See Table VI and Figure 4.) As the rate of oxygen uptake was still increasing at the highest concentration tested, the saturation concentration for arabinose cannot be determined from the data obtained. The rate of oxygen uptake at 0.800 millimole of arabinose was smaller than the saturation rate for glucose by a factor of 0.74 ± 0.03 . The configuration of these sugars is such that the last 3 isometric carbons of D-glucose and the last 3 isometric carbons of L-arabinose are optical isomers. Therefore, it is safe to say that the enzymes involved in arabinose oxidation are different from the enzymes involved in glucose oxidation, and furthermore, the arabinose oxidases are less active than either the glucose or xylose oxidases.

Effect of Acetate Concentration on the Rate of Oxygen Uptake by Pseudomonas Fluorescens. For 3.0 milligrams of Pseudomonas fluorescens suspended in 2.5 milliliters of 0.05 molar phosphate buffer of pH 6.8 and at a temperature of 25 °C the effect of acetate concentration on the oxygen uptake is quite different to the effect of the sugars studied. The rate of oxygen uptake increases with acetate concentration from concentrations of 0.0008 to 0.0250 millimole per test, remains essentially constant from 0.0250 to 0.1000 millimole per test, and then decreases with increasing concentration from 0.1000 to 0.8000 millimole per test. (See Table VI and Figure 4.) These results are probably best discussed by considering the function of acetate concentration versus temperature in two separate parts: the function from 0.0008 millimole to 0.1000 millimole concentration, and the function from 0.1000 millimole to 0.8000 millimole concentration. The first portion of the curve shows a striking resemblance to the results obtained for glucose and xylose. Considering the portion of the curve between the concentrations of 0.0008 and 0.1000 millimole of acetate, the saturation concentration for acetate is approximately 0.250 millimole and the rate of oxygen uptake at saturation for acetate is less than that for glucose by a factor of 0.90 ± 0.04 . The enzymes involved in

glucose and acetate oxidation are obviously different. The curve between the concentrations of 0.1000 and 0.8000 millimole shows a decided decrease in rate of oxygen uptake with increasing concentration. The uptake at the peak concentration of 0.8000 millimole is actually negative when compared to the endogenous rate of oxygen uptake. (Endogenous rate is the rate of uptake when the cells are suspended in buffer in the absence of any substrate.) From these results it seems that under the conditions of the experiment, acetate begins to inhibit its own oxidation as the concentration increases beyond 0.1000 millimole, and inhibits all oxidative respiration when present in a concentration of 0.8000 millimole. The explanation for the phenomenon must lie in some property of the substrate rather than the enzymes. The acetate source used was sodium acetate, so the first hypothesis would be that the high salt concentration was causing an appreciable pH change. However, the pH of the solution was checked, and the buffer proved to be quite adequate in holding the pH within +0.1 unit of pH 6.8. The next possibility considered was that the acetate solution did give a high electrolyte concentration. A high electrolyte concentration could be inhibitive both from abnormally high osmotic pressures, and the high concentration of

charged particles. To test this hypothesis a special experiment was devised (see Table VI, investigation No 32) in which 0.500 millimole of sodium acetate and 0.7500 millimole of sodium chloride were used as substrate. The mixed substrate would give approximately the same ionic concentration as an 0.8000-millimole acetate substrate. The results of this test indicated that there was some inhibition, with the rate of uptake for the mixed substrate being less than the rate for 0.0500-millimole acetate substrate by a factor of 0.82 ± 0.04 . The inhibition caused by the electrolyte is quite minor compared to the negative uptakes obtained with 0.8000-millimole acetate. It therefore seems obvious that the inhibition is not caused by some general phenomenon such as pH or ion concentration, but is rather a specific phenomenon of one of the ions present. The sodium ion concentration in the mixed sodium acetate-sodium chloride substrate was approximately the same as for the 0.8000-millimole acetate determination, so this would eliminate the sodium ion as the specific inhibitor. Therefore, assuming that the inhibition is specific, the acetate ion must be the specific inhibitor. The possibility of this specific inhibition of acetate ion is further demonstrated by the work of Jezeski et al⁽⁴¹⁾ in which one of their cultures of the genus Pseudomonas was capable of oxidizing methyl acetate but was inhibited by sodium acetate.

Mathematical Analysis of Effect of Substrate Concentration.

For 3.0 milligrams of Pseudomonas fluorescens suspended in 2.5 milliliters of 0.05 molar phosphate buffer of pH 6.8, the rate of oxygen utilization increases with substrate concentration for glucose, xylose, arabinose, and acetate, within certain low concentration ranges. It has also been shown that when substrate concentrations are increased to 0.0500 millimole per test for glucose, 0.600 millimole per test for xylose, and 0.0250 millimole per test for acetate, no further increase in rate of oxygen utilization takes place with increasing concentration of substrate. This same phenomenon has been observed in working with pure enzyme preparations⁽¹⁰³⁾. If one then makes the assumption that one enzymatic reaction is the controlling reaction in the oxidation of substrate, the reaction kinetics should follow closely those for pure enzyme preparations. For a single enzymatic reaction, it may be assumed that the primary reaction is the formation of an enzyme-substrate intermediate, which in turn breaks down into some reaction product plus the unchanged enzyme. If it is further assumed that the rate of reaction is dependent on the concentration of intermediate, the change in rate of reaction with substrate concentration can be expressed mathematically by use of an equation known as the Michaelis-Menten equation⁽¹⁰³⁾;

$$v = \frac{VS}{K_m + S}$$

where:

v = rate of reaction, quantity per unit time

V = saturation rate or rate of reaction at the point where further increase in substrate concentration does not increase reaction rate, quantity per unit time

S = substrate concentration, concentration units

K_m = reaction constant, concentration units.

It is obvious that the values of V and K_m are characteristic of the system in question. The value of V, sometimes called the "saturation rate," is determined experimentally. By mathematical manipulation of the equation it can be shown that K_m is equal to the substrate concentration, S, when the reaction velocity, v, is equal to one-half of the saturation rate, V.

For the system in question, if reaction rates are expressed as microliters of oxygen utilized per hour, and substrate concentration is expressed as millimoles per test; for glucose the value of V is 135 microliters of oxygen per hour and K_m is 0.0047 millimole. When these values are substituted in the Michaelis-Menten equation, the average deviation of experimental

values of \underline{y} from values calculated by the equation is 6.5 per cent. For xylose the value of \underline{V} is 143 microliters of oxygen per hour and $\underline{K_m}$ is 0.055 millimole per test. Using these values in the equation the average deviation of experimental values of \underline{y} from values calculated by the equation is 5.0 per cent. For acetate the value of \underline{V} is 113 microliters of oxygen per hour and $\underline{K_m}$ is 0.0005 millimole. With these values the average deviation of experimental values of oxygen utilization from values calculated by the equation is 10.0 per cent.

In the case of arabinose, the rate of oxygen uptake was still increasing with concentration at the highest concentration (0.800 millimole per test) tested. Therefore, it was impossible to obtain a value of \underline{V} from the data. However, it was possible to substitute values of \underline{y} and \underline{S} into the equation for various tested concentrations, and by solving the resulting equations simultaneously, values were calculated for \underline{V} and $\underline{K_m}$. The best value found for \underline{V} was 176 microliters of oxygen per hour and $\underline{K_m}$ 0.680 millimole. With these values, the average deviation of experimental values of \underline{y} from values calculated by the equation is 5.5 per cent.

In the case of acetate, as the concentration of substrate was increased beyond 0.1000 millimole per test, to 0.8000 millimole per test, the rate of oxygen utilization decreased with concentration. At 0.1000 millimole the rate of oxygen consumption was 115 microliters per hour. At 0.2000 millimole the utilization rate dropped to 73 microliters per hour, a drop of 42 microliters. At 0.400 millimole the rate was 27 microliters per hour, a drop of 88 microliters from the initial rate. At 0.8000 millimole the rate was - 17 microliters per hour, a drop of 132 microliters from the initial rate. It can be seen that as concentration increased in the ratio of 1:2:4:8, the net loss in rate increased in the ratio of 0:1:2:3, where the average loss is 44 microliters per hour. By means of these two ratios it is possible to express the rate of oxygen utilization as a function of substrate concentration by means of the following equation:

$$v = 115 - 44 \frac{\log_{10} S}{\log 2} = 115 - 146 \log_{10} S$$

where:

v = rate of oxygen uptake, microliters per hour

S = substrate concentration, millimoles per test.

The average deviation of experimental values of \underline{v} from the values calculated by the equation is 0.7 per cent.

Effect of Concentration of Acetate and Glucose on the Rate of Oxygen Uptake by Bacillus Subtilis. A series of experiments on the effect of concentration of acetate and glucose on the rate of oxygen uptake by Bacillus subtilis were performed by Ketner⁽⁴²⁾ who was a collaborator. Unfortunately, the concentration range studied was only from 0.0125 to 0.1000 millimole of substrate per test. Over this range of concentration the rate of oxygen uptake was essentially constant and independent of substrate concentration. (See Table VIII and Figure 6.) The slight rise in the rate of oxygen uptake with glucose at 0.0250 millimole concentration is within experimental error and probably not significant. In general, the only information that can be obtained from these data is that for 2.8 milligrams of Bacillus subtilis suspended in 2.5 milliliters of 0.05 molar phosphate buffer at a temperature of 25 °C, the saturation concentration of both glucose and acetate is below 0.0125 millimole of substrate per test.

Effect of Temperature on the Rate of Oxygen Uptake by Pseudomonas Fluorescens. In studying the effect of temperature on the rate of oxygen uptake by Pseudomonas fluorescens all variables other than environmental temperature were held constant, so the effect observed is a true temperature effect. With 3.0 milligrams of Pseudomonas fluorescens suspended in 2.5

milliliters of 0.05 molar phosphate buffer of pH 6.8, the rate of oxygen uptake on D-glucose, D-xylose, L-arabinose and acetate all increased with temperature over the temperature range from 15 to 37 °C. For a concentration of 0.1000 millimole per test glucose, the rate of uptake increased from 44 microliters per hour at 15 °C to 286 microliters per hour at 37 °C. For 0.400 millimole of xylose per test, the rate increased from 40 microliters per hour at 15 °C to 194 microliters per hour at 37 °C. With an arabinose concentration of 0.400 millimole per test the rate of uptake increased from 32 microliters per hour to 80 microliters per hour going from 15 to 37 °C. For a concentration of 0.0500 millimole of acetate per test the rate of uptake increased from 43 microliters per hour at 15 °C to 230 microliters per hour at 37 °C. (See Table VII.) If the rate of oxygen uptake is plotted against temperature for glucose, xylose, arabinose and acetate, the resultant curves are all sigmoid in shape. Although it would be possible to approximate a smooth curve through the experimental points, it is felt that straight lines would represent the plot of the function just as well as a curve within the accuracy of the data obtained. Assuming that the function is linear between inflection points, the slope of the temperature versus rate of oxygen uptake curve for glucose is 15.8 microliters per hour per

degree centigrade between 15.0 and 20.0 °C, 2.4 microliters per hour per degree centigrade between 20.0 and 25.0 °C and 12.6 microliters per hour per degree centigrade between 25.0 and 37.0 °C. For xylose, the slope of the curve is 12.4 microliters per hour per degree centigrade between 15.0 and 19.0 °C, 4.2 microliters per hour per degree centigrade between 19.0 and 35.0 °C and 16.2 microliters per hour per degree centigrade between 35.0 and 37.0 °C. For arabinose the slope of the curve is 10.4 microliters per hour per degree centigrade between 15.0 and 17.5 °C, 0.4 microliter per hour per degree centigrade between 17.5 and 30.0 °C and 2.4 microliters per hour per degree centigrade between 30 and 37 °C. For acetate the slope of the curve is 19.7 microliters per hour per degree centigrade between 15.0 and 18.0 °C, 2.4 microliters per hour per degree centigrade between 18.0 and 27.5 °C and 11.1 microliters per hour per degree centigrade between 27.5 and 37.0 °C. (See Figure 5.) Since the plots of temperature versus rate of oxygen uptake are similar in shape for all four substrates, they will be discussed together.

The shape of the temperature-reaction rate curve is not that which would be predicted from any theory on the effect of temperature on rate of reaction. However, it must be remembered that the biological oxidation of these substrates is not a single reaction

but rather a complex series of reactions. The reactions are catalyzed by colloidal catalysts, the enzymes. Furthermore, since the reactions are oxidations they are probably taking place intracellularly. Therefore, in addition to the fact that temperature changes can affect the activity of the substrate, hydrogen acceptor and the enzymes, it is possible that such physical effects as changes in environmental viscosity, changes in the viscosity of the cell contents, changes in cell wall permeability and changes in interfacial tension between cell wall and environment might easily affect the over-all rate of reaction.

One important aspect in the interpretation of these results is the temperature effect on the enzymes involved. The enzymes themselves are quite labile compounds and under normal conditions they are eventually inactivated in use. As a general rule the speed of inactivation increases with temperature. In enzymatic reactions there are two competing effects as temperature increases. The rate of reaction is increased, causing more substrate to be reacted, but at the same time the rate of inactivation of the enzyme increases, thereby lowering the concentration of the catalyst. It is evident that in specifying the optimum temperature for an enzymatic reaction it is necessary to specify

the time interval over which the reaction will be carried out. For example, in this investigation, with observation periods of one hour, the rate of oxygen uptake was greatest at 37 °C. However, if observations had been made for 24 hours, it is quite possible that the enzymes would have been completely inactivated in 3 hours at 37 °C while those at 25 °C might have retained their full activity for the 24-hour period. In this hypothetical case, if glucose were the substrate, the organisms would have consumed 4 times as much oxygen at 25 °C as they did at 37 °C for the 24-hour period. The optimum temperature for the 24-hour observation would then have been much closer to 25 °C than 37 °C. From the viewpoint of the organism itself, the optimum temperature would be that temperature at which the rate of reaction was relatively high, and the rate of enzyme inactivation was lower than the rate of cellular enzyme synthesis.

One possible biological interpretation for the data obtained and for the sigmoid shape of the temperature versus the rate of oxygen uptake curves might be as follows: at temperatures below the first inflection point in the curve, the rate of reaction and the rate of inactivation is slow, as is the rate of cellular growth and synthesis. As the temperature increases, the rate of reaction and also the rate of inactivation increase, and as the

first inflection point in the curve is passed, some apparent control on the rate of reaction takes place. The lowering in rate of reaction would almost certainly mean a lowering in the rate of enzyme inactivation, which would allow the synthetic process to keep ahead of the destructive process. As the temperature continues to increase, the synthetic ability of the organisms increases up to some optimum temperature which would be close to the optimum growth temperature (30 °C for Pseudomonas fluorescens). The second inflection point in the curve, or the point at which control over the rate of enzymatic reaction appears to be lost occurs at 25 °C for glucose, 35 °C for xylose, 30 °C for arabinose and 27.5 °C for acetate. These temperatures are all close to the optimum growth temperature for the organism involved, and are probably close to the optimum synthesis temperatures for the enzymes involved. The exact reason for the loss of control is not known, but it is a general biological phenomenon that when the body temperature of an organism is raised above its natural optimum by any factor, the organism will usually "burn itself out" and will die as a result.

The enzyme sensitivity at higher temperatures is very likely the cause of the 12.6 per cent deviation in experimental results noted for glucose and the 8.5 per cent deviation noted for

acetate at 35 °C. In performing the experimental investigation, the lower temperatures, from 15 to 35 °C were investigated first, and the cellular suspensions used were kept in cold storage prior to use for periods as long as 5 hours. This storage practice proved quite successful and very little loss of cellular activity was noted for the stored suspensions. However, when tests were made at 35 °C, deviations as great as 50 per cent were noted between results for fresh suspensions and stored suspensions. Experimentation finally indicated that cells stored for periods longer than 2 hours showed a decrease in activity at test temperatures of 35 °C. When experimental results were analyzed, data obtained for suspensions stored from 1 to 3 hours were used to calculate the average uptake at 35 °C. When tests were made at 37 °C the organisms used were never stored for more than 2 hours, and deviations for the experimental results for glucose and acetate are less than 3 per cent. (See Table VII.)

One temperature factor that was not investigated was the effect of temperature on the rate of oxygen uptake as a function of concentration of substrate. It is quite possible that the saturation concentration of the substrates, and also the concentration of acetate required for inhibition of oxygen uptake may vary with temperature. Since no data are at present available for

the effect of temperature on the rate of oxygen uptake as a function of concentration of substrate, this phenomenon cannot be discussed at this time. The investigation of the phenomenon should serve as the basis for a considerable extension of the work presented in this thesis.

Effect of Temperature on the Oxygen Uptake of *Bacillus*

Subtilis. Ketner⁽⁴²⁾ investigated the effect of temperature on the oxygen uptake of *Bacillus subtilis*. In his investigation 2.8 milligrams of bacteria were suspended in 2.5 milliliters of 0.05 molar phosphate buffer of pH 6.8, and substrates used were glucose and acetate. Substrate concentration for both substrates was 0.0500 millimole per test. The temperature range investigated was from 20 to 30 °C. The rate of oxygen uptake on glucose varied from 23.0 to 78.6 microliters per hour from 20 to 30 °C. The rate of oxygen uptake on acetate increased from 4.8 to 41.6 microliters per hour between 20 and 25 °C, and decreased from 41.6 to 36.0 microliters per hour between 25 and 30 °C. (See Table IX.) The rate of change of the rate of oxygen uptake on glucose was 13.6 microliters per hour per degree centigrade from 20.0 to 22.5 °C and 2.9 microliters per hour per degree centigrade from 22.5 to 30.0 °C. The rate of change of the rate of oxygen uptake with temperature for acetate was an

increase of 18.7 microliters per hour per degree centigrade from 20.0 to 21.0 °C, an increase of 4.5 microliters per hour per degree centigrade from 21.0 to 25.0 °C, and a decrease of 1.1 microliters per hour per degree centigrade from 25.0 to 30.0 °C. The effect of temperature on the oxygen uptake of Bacillus subtilis respiration on glucose between 20.0 and 30.0 °C is similar to the results obtained for Pseudomonas fluorescens respiration on glucose between 15.0 and 25.0 °C. The interpretation of the data for the respiration of the two organisms on glucose could logically be considered to be the same. The data for Bacillus subtilis on acetate between the temperatures of 20.0 and 25.0 °C also resembles the data obtained for Pseudomonas fluorescens on acetate between the temperatures of 15.0 and 27.5 °C. However, between the temperatures of 25.0 and 30.0 °C, Bacillus subtilis seems to show a decrease in rate of oxidation of acetate with increasing temperature. Although the difference is rather small, it was carefully checked and is probably significant. The effect can best be interpreted in view of existing knowledge as another example of the inhibitive effect of acetate ion. If this is so, inhibition by acetate ion must be dependent on temperature as well as concentration. However, there are many instances of increased cellular and enzyme sensitivity with temperature, so the assumption seems logical.

Recommendations

In the material that follows a number of recommendations for future work have been made. These recommendations are restricted to studies of the oxidation and utilization of D-glucose, D-xylose, L-arabinose, and acetate by Pseudomonas fluorescens. It is quite obvious that these same studies could and should be made with other substrates and other organisms. It is also the belief of the author that when sufficient pure culture-single substrate data have been made available, studies should be performed with systems involving pure culture-mixed substrate, mixed culture-single substrate and mixed culture-mixed substrate. When these last mentioned studies are complete and correctly interpreted, the engineer and biologist will be aided considerably in making intelligent evaluations of organic wastes and in designing and devising sound methods for the elimination of the organic waste problems.

Determination of Growth Rates. As a result of this investigation, information is available on the rate of oxygen uptake by Pseudomonas fluorescens when oxidizing D-glucose, D-xylose, L-arabinose and acetate both as a function of temperature and substrate concentration. However, if one wished to evaluate these compounds as pollutants, it would also be necessary to

know how they affect the rate of growth. It is recommended that studies be made on the effect of D-glucose, D-xylose, L-arabinose and acetate on the rate of growth of Pseudomonas fluorescens. The effect of these compounds on growth should be studied as a function of temperature of environment, pH of environment, and concentration of substrate. The studies could be made using an inorganic salts medium and adding the substrate desired to this medium. Data for the rate of growth could be taken nephelometrically.

Determination of Rate of Oxygen Uptake During Growth. The data presented in this investigation give the rate of oxygen uptake for resting cells of Pseudomonas fluorescens on glucose, xylose, arabinose and acetate. To apply these data to natural conditions it would be necessary to have information not only on the rate of growth, but also on the oxygen requirements for growth in excess of respirational oxygen requirements. It is recommended that studies be made on the rate of oxygen uptake by Pseudomonas fluorescens during growth in the presence of D-glucose, D-xylose, L-arabinose and acetate. The manometric technique suggested by McLean and Fisher⁽⁴⁸⁾ could be employed. In this method a small amount of some nitrogen source is added to a buffered cellular suspension which is already respiring on some carbon source. The rate of oxygen uptake is followed manometrically until the nitrogen

is exhausted and growth ceases. The data can be analyzed to give the percentage of the oxygen uptake required for growth and the percentage required for respiration in a growing culture. The results will vary somewhat with nitrogen source, but determinations with nitrate nitrogen, ammonia nitrogen, and organic nitrogen in the form peptone should give a good idea of the range of values to be expected.

Determination of the Effects of pH on the Rate of Oxygen Uptake. In this investigation all oxygen uptakes were determined at a pH of 6.8. Since pH may very likely affect the rate of oxygen uptake, it is recommended that studies be made to determine the effect of pH on the rate of oxygen uptake by Pseudomonas fluorescens respiration on D-glucose, D-xylose, L-arabinose and acetate. The pH range studied should be from 5 to 9, which would represent the extreme limits for most bacteria. It is suggested that tests be made in increments of 0.2 pH units over the range studied. The data could be obtained manometrically and studies should be made with both resting and proliferating cells.

Determination of the Effect of Buffer Concentration on the Rate of Oxygen Uptake. In a recent report Jezeski et al.⁽⁴¹⁾ have shown that buffer concentration can affect the rate of oxygen uptake of bacteria. This phenomenon is probably not of too great importance under natural conditions, but should nevertheless be

investigated. It is recommended that a series of tests be performed to determine the effect of buffer concentration on the rate of oxygen uptake by Pseudomonas fluorescens respiration on D-glucose, D-xylose, L-arabinose and acetate. It is suggested that a phosphate buffer of pH 6.8 be used and that tests be conducted over a concentration range of 0.02 to 0.12 molar phosphate ion. Tests should be made at increments of 0.02 molar concentration units. Data for oxygen uptake could be taken manometrically.

Determination of Substrate Saturation Concentrations at Various Temperatures. In this investigation the saturation concentrations (the lowest concentration of substrate at which further increase in concentration will not increase the rate of reaction) for D-glucose, D-xylose and acetate was determined at 25 °C. It is not known if the value of the saturation concentration varies with temperature. It is recommended that a series of tests be made to determine the saturation concentration for D-glucose, D-xylose and acetate being oxidized by Pseudomonas fluorescens at 15, 20, 30 and 35 °C. The data could be determined manometrically.

Determination of the Effect of Bacterial Concentration on the Rate of Oxygen Uptake at Various Temperatures. In this investigation the rate of oxygen uptake by Pseudomonas fluorescens as a function of temperature was determined over the temperature

range of 15 to 37 °C. In all these determinations the bacterial concentration was 3.0 milligrams of dry bacterial cells per test. It seems logical to assume that the rate of change in rate of oxygen uptake with temperature would not vary with cellular concentration. However, this is quite important and should be checked experimentally. It is recommended that a series of tests be performed to determine the change in rate of oxygen uptake by 1.0, 1.5, 2.0 and 2.5 milligrams (dry weight) of Pseudomonas fluorescens respiration of D-glucose, D-xylose, L-arabinose and acetate at 15, 20, 25, 30 and 35 °C. Data for the rate of oxygen uptake should be taken manometrically.

Determination of Acetate Inhibition as a Function of Temperature. In the study of the effect of acetate concentration on the rate of oxygen uptake by Pseudomonas fluorescens it was noted that acetate began inhibiting its own oxidation at a concentration of approximately 0.1000 millimole per test. The effect was studied only at 25 °C. It is recommended that the effect of acetate concentration on the rate of oxygen uptake by Pseudomonas fluorescens be studied at 15, 20, 30 and 35 °C. These studies would indicate whether or not the inhibition concentration changes with temperature.

Effect of Physical Changes in Cultural Environment on the Rate of Oxygen Uptake by Pseudomonas Fluorescens. Changes in various physical factors during laboratory culture, and the previous history of an organism under natural conditions could affect the rate of oxygen uptake of the organism on any given substrate. Physical factors which might affect the oxygen uptake of test organisms are temperature of culture medium and pH of culture medium. It is recommended that these factors be studied. Tests to study the effects of culture temperature could easily be devised by incubating the test organisms at temperatures from 20 to 35 °C. Temperatures could be changed to 5 °C increments. The effect of pH of the medium could be studied by varying the pH over the range of pH 6 to pH 8 at 0.5 pH unit increments.

Effect of Chemical Variation in Cultural Environment on the Rate of Oxygen Uptake by Pseudomonas Fluorescens. Chemical factors that might affect the oxygen uptake of the test organisms are the oxygen content of the culture medium and the composition of the culture medium, particularly if the medium contains the test substrate. It is recommended that these two factors be investigated. Tests to determine the effect of oxygen content could be devised by placing atmospheres of varying oxygen composition over the media, and then agitating the media during culture at a

rate sufficient to assure saturation with oxygen. The effect of the presence of the test substrate during culture could be tested by adding the substrates of D-glucose, D-xylose, L-arabinose and acetate to the nutrient broth in the amount of 50 grams of substrate per liter of broth.

Determination of Total Oxygen Uptake. The total oxygen uptake by Pseudomonas fluorescens when oxidizing D-glucose, D-xylose, L-arabinose and acetate was not determined in this investigation. If the rate of uptake and total uptake in terms of mols of oxygen per mol of substrate were known, the time required to oxidize a definite amount of substrate could be estimated. It is recommended that the total oxygen uptakes be determined. Experimentally, this would merely mean an extension of the oxygen uptake tests until the substrate was exhausted.

Determination of Rate of Carbon Dioxide Evolution and Total Carbon Dioxide Evolution During Oxidation. A knowledge of the rate of evolution and the total evolution of carbon dioxide by Pseudomonas fluorescens when oxidizing D-glucose, D-xylose, L-arabinose and acetate would give a good indication as to the rate of removal and completeness of removal of oxidizable carbon atoms. It is recommended that tests be made to determine the rate of carbon

dioxide evolution and total carbon dioxide evolution by Pseudomonas fluorescens. These tests could be made using the manometric techniques outlined by Umbreit et al⁽¹¹⁴⁾. These tests should be made with both resting cells and proliferating cells.

Determination of Carbon Balance. It is recommended that attempts be made to make a carbon balance over the system when Pseudomonas fluorescens is oxidizing D-glucose, D-xylose, L-arabinose and acetate. These data can be determined as follows: the total carbon dioxide is determined manometrically. The increase in cellular carbon and the residual carbon in solution are determined by micro-combustion methods. The sum of the weights of carbon dioxide carbon, increased cellular carbon, and residual carbon should equal the weight of the carbon added in the substrate.

Determination of Products of Oxidation. Assuming that there is residual solution carbon left after the oxidation of D-glucose, D-xylose, L-arabinose and acetate by Pseudomonas fluorescens, it would be important to know what compounds the carbon remained in. It is recommended that this determination be made by allowing the organism to completely oxidize its

substrate in 200 or 300 milliliters of solution, and then subject the remaining solution to qualitative and quantitative organic analysis.

Limitations

The following limitations were imposed on this investigation:

Organism Used. Only one species of bacteria, Pseudomonas fluorescens was used in this investigation. Standard aseptic techniques were used throughout to assure the purity of the culture. This organism was chosen because it is a common water organism, quite hardy, and strictly aerobic in nature.

Culture Medium. The test organisms used in this investigation were all cultured in 400 milliliters of nutrient broth contained in a 1,000-milliliter erlenmeyer flask.

Incubation Temperature. The cells of Pseudomonas fluorescens used in this investigation were all cultured at a temperature of 30 ± 2 °C. This temperature was chosen because the organism is stated to have an optimum growth temperature of 30 °C.

Harvest Time for Cells. The cells used in this investigation were all harvested after 20 to 24 hours of growth. This harvest time was chosen because the cells were known to be actively growing and in the logarithmic phase of their growth after 20 hours of culture.

Analysis of Concentration of Bacterial Suspensions. The concentration of the bacterial suspensions used was determined by nephelometric analysis. This method was chosen because it has proven accurate in previous investigations, and is much more rapid than counts or weighing of cells, and is more accurate than measuring the volume of packed wet cells.

Concentration of Bacterial Suspensions Used. In this investigation all tests were made with bacterial suspensions containing 1.5 milligrams of dry bacterial protoplasm per milliliter. Two milliliters of suspension were used in each test. This concentration was chosen because when acting on 0.1000 millimole of glucose it gave an uptake of 135 microliters of oxygen per hour. This rate is approximately midway between the rates of 100 and 200 microliters per hour, the stated range of greatest accuracy for Warburg manometric determinations.

Substrates Used. The substrates used to determine the oxygen uptake of Pseudomonas fluorescens were D-glucose, D-xylose, L-arabinose and ^{what acetate?} acetate. These substrates were chosen because they might be found, both free and combined, in wastes arising from chemical wood pulping, an industry which has an important waste problem.

Substrate Concentration in Tests Involving the Effect of Concentration of Substrate on the Rate of Oxygen Uptake. D-glucose and acetate were tested over a concentration range of 0.8000 to 0.0008 millimole per test. Concentrations were varied from low concentration to high concentration by a factor of 2 for each step. The D-xylose and L-arabinose were tested over a concentration range from 0.100 millimole to 0.800 millimole per test. Concentrations were varied from low concentration to high concentration by a factor of 2 for each step. The upper limit of the concentrations was determined in the case of sugars by the accuracy of pipetting of the rather viscous solutions, and in the case of acetate by the fact that an 0.8000-millimole acetate concentration completely inhibited oxygen uptake. The lower limit was determined by the reliable accuracy of making up test solutions and by the accuracy of the manometric determinations, or in some cases by both of these factors.

Substrate Concentrations in Tests on the Effect of Temperature on the Rate of Oxygen Uptake. The concentration of D-glucose used in tests on the effect of temperature on the rate of oxygen uptake by Pseudomonas fluorescens was 0.1000 millimole per test. The concentration of acetate was 0.0500 millimole per test. The concentration of D-xylose was 0.400 millimole per test. The concentration

of L-arabinose was 0.400 millimole per test. The concentrations of glucose and acetate were chosen because they were well within the saturation concentration for these substrates as determined at 25 °C. The concentrations of xylose and arabinose were chosen primarily because solutions of this concentration were not appreciably viscous and could be easily and accurately pipetted.

Temperatures Investigated. The temperatures used in tests on the effect of temperature on the oxygen uptake by Pseudomonas fluorescens were 15.0, 17.5, 20.0, 25.0, 30.0, 35.0 and 37.0 °C. The lower temperature was determined by the physical limitations of the equipment used and the upper temperature limit was determined by the biological limits of the organisms tested.

Buffer Used. The buffer used in this investigation was a 0.05 molar phosphate buffer. The phosphate buffer was chosen because previous work has shown that phosphate buffer works very well for studies of oxygen uptake of bacteria.

pH of Tests. All tests in this investigation were done at a pH of 6.8. The choice of pH was arbitrary, the only requirement being considered was that it must be between pH 6.0 and pH 8.0. The slightly acid pH close to the center of the range was favored because of the alkaline nature of the sodium acetate substrate.

Determination of Oxygen Uptakes. Oxygen uptakes were determined by only one method, the direct Warburg technique. The Warburg technique was chosen because it is one of the most rapid methods of determining oxygen uptakes and also gives a high degree of accuracy for biological work (maximum error of 5 per cent).

Bacteriological Techniques Used in Determinations. All determinations of the oxygen uptake of Pseudomonas fluorescens were made using the resting cell technique. This technique was chosen because it allows determinations to be made with a constant bacterial concentration, making interpretation of data accurate and easy.

V. CONCLUSIONS

In the investigation of the effect of temperature and substrate concentration on the rate of oxygen uptake by Pseudomonas fluorescens, all tests were made with 3.0 milligrams of bacteria (stated as dry bacterial protoplasm) suspended in 2.5 milliliters of 0.05 molar phosphate buffer. The substrates used were D-glucose, D-xylose, L-arabinose and acetate.

The effect of concentration of substrate on the rate of oxygen uptake by Pseudomonas fluorescens were:

1. The rate of oxygen uptake by Pseudomonas fluorescens on glucose increased with glucose concentration from the value of 16 microliters per hour at 0.0008 millimole substrate to 131 microliters per hour at 0.0500 millimole substrate.

2. The rate of oxygen uptake as a function of substrate concentration can be approximated between 0.0008 and 0.0500 millimole glucose by the following adaptation of the Michaelis-Menten equation:

$$v = \frac{135 S}{0.0047 + S}$$

where:

v = rate of oxygen uptake, microliters per hour

S = substrate concentration, millimole.

The average deviation of experimental values from values calculated by the equation is 6.5 per cent.

3. The rate of oxygen uptake by Pseudomonas fluorescens on glucose was independent of substrate concentration between concentration values of 0.0500 and 0.8000 millimole, and had an average value of 131 microliters of oxygen per hour.

4. The rate of oxygen uptake by Pseudomonas fluorescens respiration of D-xylose increased with substrate concentration from the value of 84 microliters per hour at 0.100 millimole substrate to 143 microliters per hour at 0.600 millimole substrate.

5. The rate of oxygen uptake as a function of substrate concentration can be approximated between 0.100 and 0.600 millimole xylose by the following adaptation of the Michaelis-Menten equation:

$$v = \frac{143 S}{0.055 + S}$$

where:

v = rate of oxygen uptake, microliters per hour

S = substrate concentration, millimole.

The average deviation of experimental values from values calculated by the equation is 5.0 per cent.

6. The rate of oxygen uptake by Pseudomonas fluorescens respiration on D-xylose was independent of substrate concentration between concentration values of 0.600 and 0.800 millimole and had an average value of 143 microliters of oxygen per hour.

7. The rate of oxygen uptake by Pseudomonas fluorescens on L-arabinose increased with substrate concentration from the value of 24 microliters per hour at 0.100 millimole substrate to 93 microliters per hour at 0.800 millimole substrate.

8. The rate of oxygen uptake as a function of substrate concentration can be approximated between 0.100 and 0.800 millimole arabinose by the following adaptation of the Michaelis-Menten equation:

$$v = \frac{176 S}{0.680 + S}$$

where:

v = rate of oxygen uptake, microliters per hour

S = substrate concentration, millimole.

The average deviation of experimental values from values calculated by the equation is 5.5 per cent.

9. The rate of oxygen uptake by Pseudomonas fluorescens respiration on acetate increased with substrate concentration from the value of 75 microliters per hour at 0.0008 millimole substrate to 115 microliters per hour at 0.0250 millimole substrate.

10. The rate of oxygen uptake as a function of substrate concentration can be approximated between 0.0008 and 0.0250 millimole acetate by the following adaptation of the Michaelis-Menten equation:

$$v = \frac{113 s}{0.0005 + s}$$

where:

v = rate of oxygen uptake, microliters per hour

S = substrate concentration, millimole.

The average deviation of experimental values from values calculated by the equation is 10 per cent.

11. The rate of oxygen uptake by Pseudomonas fluorescens respiration on acetate was independent of substrate concentration between concentration values of 0.0250 and 0.1000 millimole and had an average value of 116 microliters of oxygen uptake per hour.

12. The rate of oxygen uptake by Pseudomonas fluorescens respiration on acetate decreased with increasing substrate concentration from the value of 115 microliters per hour at 0.1000 millimole substrate to - 17 microliters per hour at 0.8000 millimole substrate.

13. The rate of oxygen uptake as a function of substrate concentration can be approximated between 0.1000 and 0.8000 millimole acetate by the following equation:

$$v = 115 - 146 \log_{10} S$$

where:

v - rate of oxygen uptake, microliters per hour

S - substrate concentration, millimole.

The average deviation of experimental values from values calculated by the equation is 0.7 per cent.

With substrate concentrations of 0.1000 millimole of D-glucose, 0.400 millimole of D-xylose, 0.400 millimole of L-arabinose and 0.500 millimole of acetate, the effects of temperature on the rate of oxygen uptake were:

1. The rate of oxygen uptake by Pseudomonas fluorescens respiration on glucose increased with temperature from 44 to 286 microliters per hour between 15.0 and 37.0 °C.

2. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on glucose was 15.8 ± 1.6 microliters per hour per degree centigrade from 15.0 to 20.0 °C.

3. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on glucose was 2.4 ± 0.2 microliters per hour per degree centigrade from 20.0 to 25.0 °C.

4. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on glucose was 11.7 ± 1.2 microliters per hour per degree centigrade from 25.0 to 37.0 °C.

5. The rate of oxygen uptake by Pseudomonas fluorescens on D-xylose increased with temperature from 40 to 194 microliters per hour between the temperatures of 15.0 and 37.0 °C.

6. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on xylose was 18.0 ± 1.8 microliters per hour per degree centigrade between 15.0 and 18.0 °C.

7. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on xylose was 3.9 ± 0.4 microliters per hour per degree centigrade from 18.0 to 35.0 °C.

8. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on xylose was 16.5 ± 1.6 microliters per hour per degree centigrade from 35.0 to 37.0 °C.

9. The rate of oxygen uptake by Pseudomonas fluorescens on L-arabinose increased with temperature from 32 to 80 microliters per hour between 15.0 and 37.0 °C.

10. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on arabinose was 10.4 ± 1.0 microliters per hour per degree centigrade from 15.0 to 17.5 °C.

11. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on arabinose was 0.4 ± 0.0 microliters per hour per degree centigrade from 17.5 to 30.0 °C.

12. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on arabinose was 2.4 ± 0.2 microliters per hour per degree centigrade from 30.0 to 37.0 °C.

13. The rate of oxygen uptake by Pseudomonas fluorescens on acetate increased with temperature from 43 to 230 microliters per hour between 15.0 and 37.0 °C.

14. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on acetate was 19.7 ± 2.0 microliters per hour per degree centigrade from 15.0 to 18.0 °C.

15. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on acetate was 2.8 ± 0.3 microliters per hour per degree centigrade from 18.0 to 28.0 °C.

16. The rate of change in rate of oxygen uptake by Pseudomonas fluorescens on acetate was 12.5 ± 1.3 microliters per hour per degree centigrade from 28.0 to 37.0 °C.

VI. SUMMARY

Industrial waste pollution is a problem of considerable magnitude and of great importance to modern industry. One critical aspect of waste pollution is reduction of dissolved oxygen in natural waters resulting from the oxygen uptake of bacteria while digesting organic wastes.

Fundamental information concerning the effect of various physical and chemical factors on the rate of oxygen uptake by bacteria was sought by investigating the rate of oxygen uptake by Pseudomonas fluorescens, a common water organism, as a function of the concentration of substrate and the temperature of the environment. The substrates used were D-glucose, D-xylose, L-arabinose and acetate, and temperatures investigated ranged from 15.0 to 37.0 °C.

Oxygen uptakes were determined manometrically using the direct Warburg method in conjunction with resting cell techniques. In all tests 3.0 milligrams of Pseudomonas fluorescens (stated as dry bacterial protoplasm) were suspended in 2.5 milliliters of 0.05 molar phosphate buffer of pH 6.8. Bacterial suspensions were analyzed nephelometrically using a Klett-Summerson photoelectric colorimeter.

At 25 °C, for all substrates tested, the rate of oxygen uptake is dependent on concentration in the lower concentration ranges.

In all cases, the variation in the rate of oxygen uptake with concentration may be expressed mathematically by the Michaelis-Menten equation:

$$v = \frac{VS}{K_m + S}$$

where:

v = rate of oxygen uptake, microliters per hour

V = maximum rate of oxygen uptake at saturation

concentration of substrate, a constant

value, microliters per hour

S = substrate concentration, millimole

K_m = the Michaelis constant, millimole.

For glucose between the concentrations of 0.0008 and 0.0500 millimole, the maximum rate, V , is equal to 135 microliters per hour and the constant, K_m , is equal to 0.0047 millimole within deviations of 6.5 per cent. For xylose between the concentrations of 0.100 and 0.600 millimole, the maximum rate, V , is equal to 143 microliters per hour and the constant, K_m , is equal to 0.055 millimole within deviations of 5.0 per cent. For arabinose between concentrations of 0.100 and 0.800 millimole, the maximum rate, V , was calculated to be 176 microliters per hour and the constant, K_m , was calculated

to be 0.680 millimole within deviations of 5.5 per cent. For acetate between the concentrations of 0.0008 and 0.0250 millimole, the maximum rate, V , is equal to 113 microliters per hour and the constant, K_m , is equal to 0.0005 millimole within deviations of 10 per cent.

The rate of oxygen uptake was found to be independent of concentration of substrate for the higher concentrations of glucose and xylose, and for intermediate concentrations of acetate. With glucose concentrations from 0.0500 to 0.8000 millimole the rate was 131 microliters per hour. With xylose concentrations between 0.600 and 0.300 millimole the rate was 143 microliters per hour, and with acetate concentrations between 0.0250 and 0.1000 millimole the rate was 116 microliters per hour.

When acetate concentration was varied from 0.1000 to 0.8000 millimole, the rate of oxygen uptake decreased with increasing substrate concentration as expressed by the following equation:

$$v = 115 - 146 \log_{10} S$$

where:

- v = rate of oxygen uptake, microliters per hour
- S = substrate concentration, millimole.

When substrate concentrations were held constant and temperature of the environment was varied from 15.0 to 37.0 °C, the rate of oxygen uptake increased with temperature in all cases. For glucose the rate of change of the rate of oxygen uptake was 15.8 ± 1.6 microliters per hour per degree centigrade between 15.0 and 20.0 °C, 2.4 ± 0.2 microliters per hour per degree centigrade between 20.0 and 25.0 °C, and 11.7 ± 1.2 microliters per hour per degree centigrade between 25.0 and 37.0 °C when glucose concentration was held constant at 0.1000 millimole. For xylose the rate of change of the rate of oxygen uptake was 18.0 ± 1.8 microliters per hour per degree centigrade between 15.0 and 18.0 °C, 3.9 ± 0.4 microliters per hour per degree centigrade between 18 and 35 °C, and 16.5 ± 1.6 microliters per hour per degree centigrade between 35.0 and 37.0 °C when xylose concentration was held constant at 0.400 millimole. For arabinose the rate of change of the rate of oxygen uptake was 10.4 ± 1.0 microliters per hour per degree centigrade between 15.0 and 17.5 °C, 0.4 ± 0.0 microliters per hour per degree centigrade between 17.5 and 30.0 °C, and 2.4 ± 0.2 microliters per hour per degree centigrade between 30.0 and 37.0 °C when arabinose concentration was held constant at 0.400 millimole. For acetate the rate of change of the rate of oxygen uptake was 19.7 ± 2.0 microliters per hour per degree centigrade

between 15.0 and 18.0 °C, 2.8 ± 0.3 microliters per hour per degree centigrade between 18.0 and 28.0 °C, and 12.5 ± 1.3 microliters per hour per degree centigrade between 28.0 and 37.0 °C when the acetate concentration was held constant at 0.0500 millimole.

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