EFFECT OF POUCH FILM PERMEABILITY ON THE GROWTH OF PSEUDOMONAS AERUGINOSA

By

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ABSTRACT

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Many food products experience end of shelf-life, due to problems associated with aerobic bacterial contamination. Meat products are a prime example. Growth of aerobic contaminants has been controlled in the past by using high barrier films with some success. However, the mechanism of inhibition is not well understood.

This research concentrates on the dynamic relationship of headspace gases and bacterial growth and metabolism, with respect to film permeability. Experimentation was confined to a single aerobic organism in a sealed pouch, which contains a liquid growth medium.

It was found that inhibition of growth increased with an increasing barrier film. Headspace O_2 concentration decreased in the pouches, where CO_2 concentration increased, with a concurrent decrease in the bacterial population. It appeared that the organisms altered their metabolism when stressed by O_2 depletion and CO_2 increases.

Once these dynamic relationships are further quantified, computer shelf-life predictions will be possible.

To my wife, Debbie, who made it all possible, my friends for their support and interest, and to Whiskey, who waited so patiently.

ACKNOWLEDGEMENTS

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CAL	=	Calibration
PE	=	Polyethylene (2 mil)
S	=	Saran (2 mil)
SN	=	Saran Coated Nylon (0.75 mil)
Std. Dev.	=	Standard Deviation
$\bar{x}$	=	Average

INTRODUCTION

It is well known that aerobic psychotrophic bacteria are common contaminants of prepackaged meat products (Ayres, 1959). Even under the most sanitary conditions these microorganisms cause a premature end of shelf-life due to organoleptic changes and potential health risks. It was soon realized that packaging could be instrumental in controlling the growth of these obligately aerobic microbes, by controlling the atmosphere inside the package. The easiest factor to control in this sense is the packaging material itself, since it's permeability to oxygen will mediate the oxygen available for bacterial metabolism.

In the early days of research it was thought that oxygen permeability was the prime factor determining the amount and rapidity of growth of contaminants. However, later research showed that other factors played a larger role in the growth of aerobes than first anticipated. Notably, the concentration of carbon dioxide in the headspace of the package, generated through bacterial metabolism or tissue respiration, has shown significant inhibition of Pseudomonas species.

The permeable pouch is a very interdependent dynamic system, in which the organism itself plays an integral role in the change of its environment. Initially, O_2 permeates in, because the O_2 concentration inside the pouch is lower than outside, due to vacuum packaging. A short time later, the organisms inside the package start the exponential phase

of growth, which increases it's respiratory rate, thus using more O_2. As the o_2 is metabolized, CO_2 is being produced in the package and as the CO_2 level builds up and the O_2 level decreases, this places a strain on the organisms and inhibits their growth. The gas permeability of the film affects the amount of O_2 permeating in and the CO_2 permeating out, due to an increase in CO_2 concentration in the pouch as compared to outside the pouch. Theoretically, a high barrier film would inhibit the bacteria more by letting in less O_2 and trapping CO_2, which is inhibitory. A low barrier film would not have as great of an effect.

This study will examine the effects of oxygen and carbon dioxide concentration on the growth of a single microorganism, Pseudomonas aeruginosa, a common aerobic contaminant of prepackaged meat products. Three different materials will be used, representing high and low permeabilities to oxygen and carbon dioxide. A commercial bacteriological substrate, commonly used to grow Pseudomonads, was used to negate any effect that tissue respiration might have on headspace gas concentration. Obviously, it is no easy task to untangle all the factors affecting microbial growth in permeable packages. However, this study will examine the effects of gas concentrations on the growth of a single organism under specific conditions.

LITERATURE REVIEW

Effects of O_2 and CO_2 Concentration On The Growth of Aerobic Organisms

To understand the effects of a film's permeability to gases on the microbial growth within a package, one must first have a basic understanding of the effect of the gas itself on microorganisms. This discussion will be confined to the genus *Pseudomonas* and other aerobic gram negative rods, which may act similarly to *Pseudomonas*. The gases under scrutiny are carbon dioxide and oxygen.

Coyne (1933) observed that bacterial growth on fresh fish was inhibited by a CO_2 enriched atmosphere. Optimal inhibition of bacterial growth seemed to occur at a CO_2 concentration of 40-60%. Visual observations of the fish carcasses were made to evaluate the amount of microbial growth. Haines (1933) conducted a more in depth study on the effect of carbon dioxide on *Pseudomonas* and other organisms. Several conclusions were drawn as to cause and effect. He found that at a CO_2 concentration of 10-20% the lag period of *Pseudomonas* is increased and the generation time was cut to almost one-half at 20°C. Another interesting observation was that generation time was almost doubled by 10% CO_2 when the temperature was lowered to 0°C, indicating that CO_2 inhibition is more effective at lower temperatures. It was noted that the maximum number of organisms reached was the same, independent of CO_2

concentration, it would just take longer with an increasing CO_2 concentration or lower temperature. It was previously thought (Valley and Rettger, 1927) that pH change to a more acidic condition was responsible for inhibition of bacterial growth, but Haines (1933) suggested that it was due to the action of the gas on dehydrogenases in the cells. Since the research of Coyne (1933) and Haines (1933) several studies have been conducted, which basically support their contentions on CO_2 inhibition of microbial growth. Dual studies by Gill and Tan (1979) showed that all concentrations (0-450 mmHg) were inhibitory to Pseudomonas at 30^{o} in a complex medium and maximum inhibition occurred at 250 mmHg. They also found, as did Haines (1933), that with a constant CO_2 concentration inhibition increased with decreasing temperature. King and Nagel (1975) came to similar conclusions in their research.

It has been suggested that a linear relationship exists between CO_2 concentration and generation time under controlled conditions (King and Nagel, 1967). However, it was found that the inhibitory effect of CO_2 is not permanent and cultures once exposed will return to normal growth upon return to atmospheric concentrations of CO_2 (Enfors, 1979).

The question now becomes, where does the CO_2 necessary for inhibition come from in a package? In meat packaging the initial increase in CO_2 concentration is a direct result of tissue respiration. This occurs in the first 3-5 hours after packaging. CO_2 concentration increases after that

point are primarily due to microbial growth (Gardner et al, 1976, Seideman et al, 1976 a, b). Seideman et al, (1976 a, b) also suggested that vacuum packages fit tighter and thus increase the partial pressure of carbon dioxide in the package and that any residual oxygen in the package would be available for conversion to carbon dioxide.

Clearly, from the evidence presented, CO_2 plays a very important role in the inhibition of bacterial growth. However, the previous discussion has not addressed the effect of O_2 concentration on the growth of bacteria. Boneless beef roasts were packaged in various atmospheric concentrations of oxygen and it was found that Pseudomonas species were present in high concentrations of oxygen, but not in the low concentrations (Christopher et al 1979). The results of this experiment coincide with what one would expect with respect to O_2 concentration and aerobic bacterial growth. Other studies temper this statement to a degree and show that oxygen dependency has its limits. King and Nagel (1967) found that by depleting oxygen in the atmosphere a limitation in the growth of Pseudomonas aeruginosa was not observed until more than 75% (volume/volume) of air was replaced with nitrogen. Clark and Burki (1972) endeavored to find the concentration levels of oxygen necessary to inhibit Pseudomonas. They found that the oxygen concentration could be lowered to 2% without significantly inhibiting growth. At 0.5-2% oxygen, the effect was to increase the lag time of Pseudomonas and at

concentration lower than 0.5% cell generation time was increased and cell yield at stationary phase was decreased. This research was done while maintaining a constant atmosphere, with respect to oxygen and carbon dioxide concentrations.

In reviewing the literature it becomes obvious that carbon dioxide concentration is just as important, if not more so, than oxygen concentration. Possibly the aerobic organisms can utilize oxygen that is dissolved in the substrate and do not rely on headspace oxygen until the dissolved oxygen is used up. Carbon dioxide, on the other hand, is not used in metabolism as oxygen is. It's effect is purely inhibitory. Therefore, it may be as important to look at carbon dioxide levels in dynamic systems, such as gas permeable packages.

Effect of Film Permeability

With a better understanding of how oxygen and carbon dioxide affect aerobic bacteria, it now becomes possible to discuss how that bacteria might act in a dynamic system, such as a gas permeable package. Given there are no holes or bad seals in a package, its permeability depends on the permeability of the film itself (Eustace 1981). Ingram (1962) did an extensive study on the effect of film permeability on bacterial growth and came to some interesting conclusions. He found that Pseudomonas species were predominant on beef carcasses that were loosely wrapped

with highly permeable film, while carcasses wrapped in a better barrier selected for more anaerobic organisms. In his studies he noted that aerobic organisms could grow in atmospheres that only had 1% oxygen, again suggesting that carbon dioxide is limiting growth. Films that allow carbon dioxide to escape would maintain aerobic conditions where more impermeable films would trap the carbon dioxide in the package. Thereby, he concluded that the concentration of gases in the package depended on gas diffusion through the film and metabolic activity inside the package. Also, as oxygen is depleted in the package more will permeate through the film and prolong the aerobic phase. Ingram observed, as others had, that a vacuum packaged product has an increased partial pressure of carbon dioxide due to the decreased headspace, but this tight fit can be eliminated as more carbon dioxide is produced, either by tissue respiration or bacteria growth.

Hess (1980) found that obligately aerobic spoilage flora depended chiefly upon the oxygen permeability of the film and the effectiveness of the vacuum applied. He concluded that oxygen permeation into the bags caused the growth of aerobic bacteria and he found a reduction in the growth of Pseudomonas with films of oxygen permeability less than 10 cc mil/m^2 day atm. He also found that aerobic organisms were considerably inhibited by vacuums of 0.001 - .02 atm and the growth of Pseudomonads was almost entirely suppressed with a high vacuum.

Another study conducted by Lund (1968) with sulphite treated peeled potatoes found that, when wrapped in saran and stored at 23°C for 3 days and at 6°C for 7 days, there was a decrease in oxygen concentration and an increase in carbon dioxide concentration. She also observed that they were lower bacterial counts of aerobes in the saran bags than in perforated films, which would allow gases to transfer easily in and out of the bag.

An observation made by Hannan (1962) also serves to shed more light on the dynamic relationship of permeability and biological activity. Hannan's research led him to the conclusion that the temperature coefficient of permeability is considerably less than that of biological activity, particularly respiration. In other words, permeability is not affected as greatly per degree of temperature change as bacterial respiration.

Other articles have been written as summaries of the type of research previously discussed (Dallyn et al 1973, Apple et al 1983, Finne 1982). Similar conclusions were drawn in these papers, providing supporting evidence to the previous research.

Finally, it is of interest to note that Pseudomonas aeruginosa produces one mole of CO_2 for every mole of O_2 utilized in metabolism (Nester et al, 1973).

MATERIALS AND METHODS

Materials

Three materials were chosen for this study, one with a high gas permeability constant, and the other two with a low permeability to oxygen and carbon dioxide. The materials selected were saran (2 mil), saran coated nylon (0.75 mil), and polyethylene (2 mil). These films represent samples of low and high gas permeabilities (Table 1) and all are heat sealable.

Table 1
Film Permeabilities

Film	Permeability ($cc/m^2/24$ hrs.) Oxygen	Carbon Dioxide
Saran (2 mil)	13.0	54.8
Saran coated nylon (0.75 mil)	17.0	67.8
Polyethylene (2 mil)	4,967	21,333

A silicone sealant was attached to the bags and functioned as a septum to aseptically remove or introduce materials into the bags without altering the headspace of the bag or contaminating the interior of the package.

Brain-heart infusion broth (BBL) was used as a medium inside the bags to grow the microorganisms and was chosen because of its known capability to grow cultures of Pseudomonas aeruginosa (Difco Labs). This broth was also used as a medium for starter cultures and bag innoculations. Plate-count agar (BBL) was selected as a medium for growth in enumerating microbes, utilizing a pour-plate technique

(Housler 1972). A 0.5 molar solution of phosphate buffer was used in the 99 ml dilution blanks used in the colony count procedure. (Formula in Appendix E). Pseudomonas aeruginosa ATCC #27853 was selected as the organism for the study, because of its aerobic requirements for adequate growth (Buchanan). A Super-Vac machine (Smith Equip. Co., Clifton, N.J.) provided the ability to draw a vacuum on the bags and make the final seal of the package after the broth was added. For headspace measurements of oxygen and carbon dioxide a Carle 8700 gas chromatograph was utilized for quantification (see Table 2 for conditions) and BBL Gas-Pak jars were used to keep an anaerobic atmosphere for the control culture.

Table 2

Carle 8700 gas chromatograph conditions

Oven temp.	-	60°C
Attenuation	-	16
Chart Speed	-	1 inch/minute
mV response	-	10mV
Columns	-	Pora-Pak and Molecular Sieve

Methods

All film samples were formed into 8"x6" pouches using an impulse sealer to heat seal three sides of the package. All pouch fabrication was performed in a laminar flowhood to avoid contamination of the interior of the bags. For sealing conditions see Table 3.

Table 3

Sealing Conditions

Film	Heat	Gas	Vacuum
Saran (2 mil)	6.0	0	3.0
Saran coated nylon (.75 mil)	4.0	0	1.5
Polyethylene (2 mil)	6.0	0	1.5

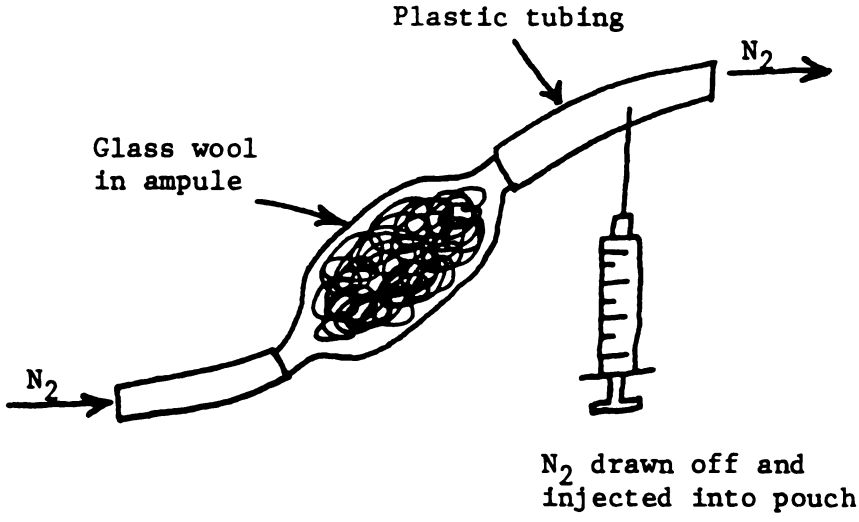
Following fabrication, brain-heart infusion broth was aseptically added to the pouches and they were vacuum packaged using the Super-Vac machine. See Table 4 for settings.

Table 4

Super-Vac Sealing Conditions

Film	Heating (sec.)	Cooling (sec.)	Pressure (p.s.i.)
Saran (2 mil)	0.35	1.5	30
Saran coated nylon (.75 mil)	0.35	1.0	30
Polyethylene (2 mil)	0.25	1.0	30

This reduced the amount of oxygen in the headspace, requiring the microorganisms to rely partially on oxygen permeating through the film for growth. After this was completed 20 cc of nitrogen was injected into the bags through the silicone septum for headspace, as shown in Figure 1. Once the nitrogen was added to the headspace the bags were incubated at 32°C for 24 hours and visually inspected for any contamination that may have occurred during pouch fabrication. Contaminated bags were rejected and only sterile ones used.



Apparatus was sterilized before use.

Figure 1

Nitrogen Injection Apparatus

A starter culture of Pseudomonas aeruginosa ATCC #27853 was prepared by inoculating 20 ml of brain-heart infusion broth with a mature culture 16 hours before use to ensure that the starter culture would be in exponential phase when needed. Before the culture was used it was serially diluted using a phosphate buffer of pH 6.5 to obtain an inoculum that was approximately 10^3-10^4 cells/ml. At this point the inoculum could be injected into each pouch for the start of the test. Plate counts were also done to determine the exact initial count of organisms to be inoculated.

Each material was tested in triplicate, making a total of nine pouches tested for all the materials. An inoculum of 1 cc of starter culture was injected into each of the

test bags. Once injected with inoculum the bags were immediately tested for headspace gasses on the Carle 8700 gas chromatograph. The pouches were then incubated at 32°C in a horizontal position in order to maximize the headspace/media interface. Colony counts and headspace measurements were taken at 0, 6 and 12 hours and thereafter as indicated by the growth rate of the organism which was a function of the permeability of the material being used.

Colony counts were performed by aseptically removing a 1 cc sample from the pouch using a syringe and then serially diluting the sample in 99 ml phosphate buffer dilution blanks. Either a 1 ml or 0.1 ml aliquot was removed from each bottle (depending on dilution required) and aseptically transferred to a petri dish, which was then filled with molten plate count agar and gently swirled to mix before hardening. Duplicate aliquots were taken for each dilution and the average of the two used for the colony count. Only plates with 30-300 colonies were counted after 24 hours of incubation at 32°C. Colony morphology of the plates was also noted, so that any contaminating organism might be detected.

Headspace measurements were taken on the Carle 8700 gas chromatograph using a 1 cc sample, removed aseptically from the pouch with a syringe. Due to the number of measurements taken and the limited amount of headspace available, only one sample was taken per pouch and no gas replaced since the pouch was flexible and would conform to the missing volume.

This ensured that alteration of the individual gas partial pressures would be minimized.

Aerobic and anaerobic control runs were synchronized with the beginning of the bag runs and inoculum for the controls were taken from the same starter culture that was used for the pouches. The aerobic control consisted of placing 20 ml of brain-heart infusion broth in a sterile petri dish and adding 1 cc of starter culture, prediluted as was done for the pouches. The petri dish was then incubated 32°C. The anaerobic control was similarly treated, except that incubation at 32°C was done in a BBL Gas-Pak jar, which provided the anaerobic atmosphere necessary. This device has a generator and catalyst which produces hydrogen and converts the oxygen in the atmosphere to water. The controls were sampled for cell concentration at the same time intervals as the pouches, using the same pour plate technique.

Permeability Measurements of Films

A MoCon Oxtran 100 was used to measure the oxygen transmission rates of the films (Modern Controls, Elk River, MN). This device utilizes the isostatic test method, which means that a constant total pressure is maintained on both sides of the film. A schematic representation of the Oxtran 100 is shown in Fig. 2.

A film sample is clamped into the diffusion cell, exposing a 100 cm^2 area of material. An oxygen-free carrier

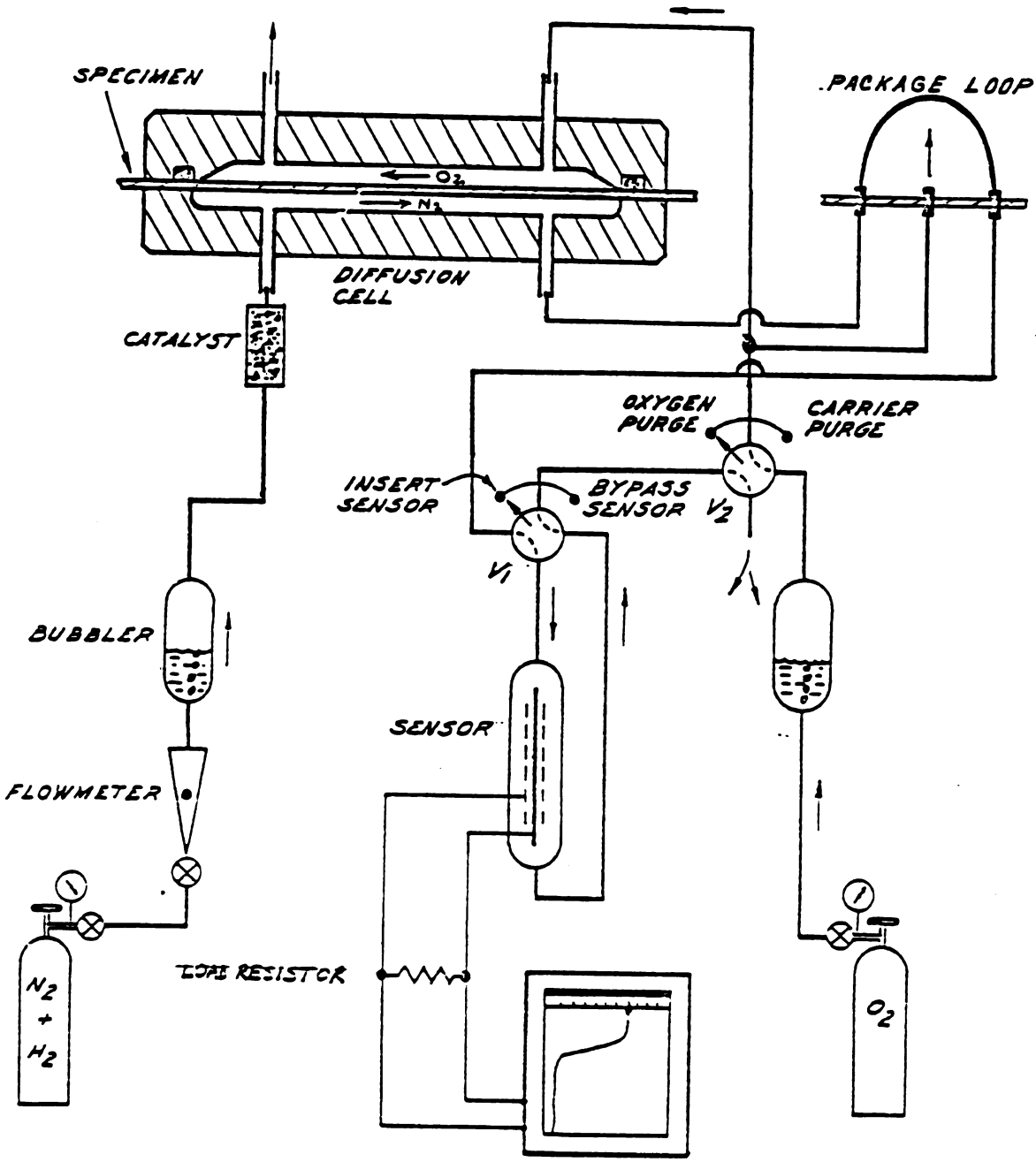


Figure 2.

MoCon Oxtran 100

gas is continually flushed over both sides of the film to remove residual oxygen. Once an oxygen-free baseline has been established, oxygen is exposed to one side of the film, while carrier gas flows over the other side and into the coulometric detector.

The coulometric detector is a constant current generator, the output of which is a linear function of the mass flow rate of oxygen entering the detector. At the surface of a graphite cathode each oxygen molecule reacts to capture four electrons.

$$O_2 + 2\ H_2O + 4e^- \text{ -- } 4OH^-$$

These hydroxide ions react at the cadmium anode to form cadmium hydroxide and release the four electrons.

$$2Cd + 4OH^- - 4e^- \text{ -- } Cd(OH)_2$$

Each molecule of oxygen causes the transfer of four electrons, therefore one mole of oxygen equals four Faradays.

1 Faraday = 96,500 ampere-seconds therefore, one mole oxygen = 4 x 96,500 = 3.86×10^5 ampere-seconds, at S.T.P. 1 cc of oxygen/24hr. = 1.99×10^{-4} amperes.

As oxygen starts to permeate through the film the mV response increases and is displayed on the chart recorder. This increase will continue until a steady state is reached, which represents the equilibrium oxygen transmission rate of the sample. The oxygen transmission rate depends on the resistor used.

5.3 Ohm: 1mV = 100cc/day/atm

53 Ohm: 1mV = 10cc/day/atm

The films were tested at 32°C and 0% RH.

A MoCon Permatran-C was used to measure carbon dioxide transmission rates of the films, utilizing the isostatic test method. The Permatran-C is similar to the Oxtran 100, except the detector system consists of a pressure modulated infrared (PMIR) concept. Instead of using a mechanical chopper to modulate the light beam, it modulates the gas pressure at a fixed frequency. Pressure is achieved by means of a metal bellows, which compresses the gas at a cyclic rate of 25-30 Hz. Since pressurized gas absorbs more infrared radiation than non-pressurized gas, the pressure waves act to modulate the infrared beam. An optical band-pass filter is used to limit the light passing through to 4.3 um, which is the wavelength associated with stretching and bending of C=O bonds found in carbon dioxide. The amplitude of the modulated signal is an indication of how much carbon dioxide is present. The signal from the detector is amplified and fed into the chart recorder.

One side of the film is exposed to 100% carbon dioxide, while the other side is purged with a stream of air passed through a bed of ascarite. The sample is allowed to condition for a period of time, during which time a uniform diffusion rate is established. Once equilibrium is reached, a valve allows the air stream to bypass the ascarite, circulate in a closed loop, past one side of the sample, into the sensing chamber and back to the sample again. As

the permeant diffuses through the sample, it accumulates in the capture volume, which is recorded on the strip chart recorder. The slope of the line made on the chart paper represents the change in volume per change in time, which is proportional to the diffusion rate through the film.

To determine what the recorder deflection means in terms of cc's of carbon dioxide, a known volume of carbon dioxide is introduced into the calibration loop and the deflection is observed. Several injections from the calibrating loop allows for the construction of a calibration curve of volts versus volume. A schematic of the Permatran-C is shown in Figure 3.

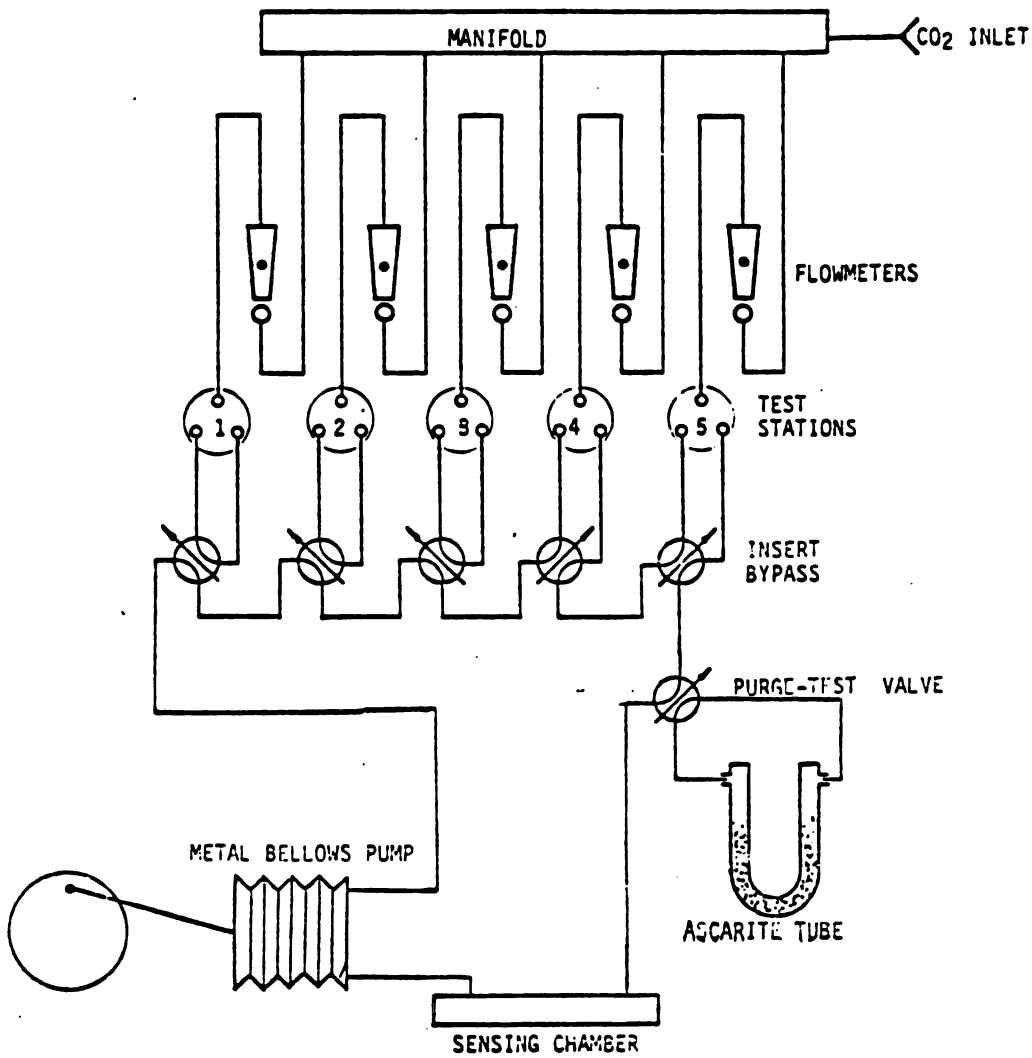


Figure 3.

MoCon Permatran-C

RESULTS

Headspace measurements and colony counts are presented in Appendices A and B, respectively. The results of which are summarized in Tables A4-6 and B4-6 and are used to construct the graphs for each material tested (Figures 4, 5 and 6). Standard deviations and statements of precision should be noted and considered when the Results section of this text is read.

In Figure 4 it is evident that the O_2 concentration initially starts to rise, due to the partial pressure difference between the inside and outside of the package. During this time the bacterial culture is progressing into exponential phase, which causes a drop in O_2 concentration in the headspace immediately thereafter, along with an increase in CO_2 concentration. The O_2 and CO_2 concentrations seem to decrease and increase respectively, until they reach a similar value. Compared to the aerobic and anaerobic controls the culture appears to be only slightly inhibited, with respect to total all yield, by the O_2 and CO_2 permeability of the film.

The saran and saran coated nylon films (Figures 5 and 6) show similar results, most likely due to their similar gas permeabilities. Saran and saran coated nylon also exhibit a concurrent decrease in headspace O_2 with an increase in bacterial growth. Also, the headspace gases approach a value, which starts when the culture approaches stationary phase. The bacterial culture, was more

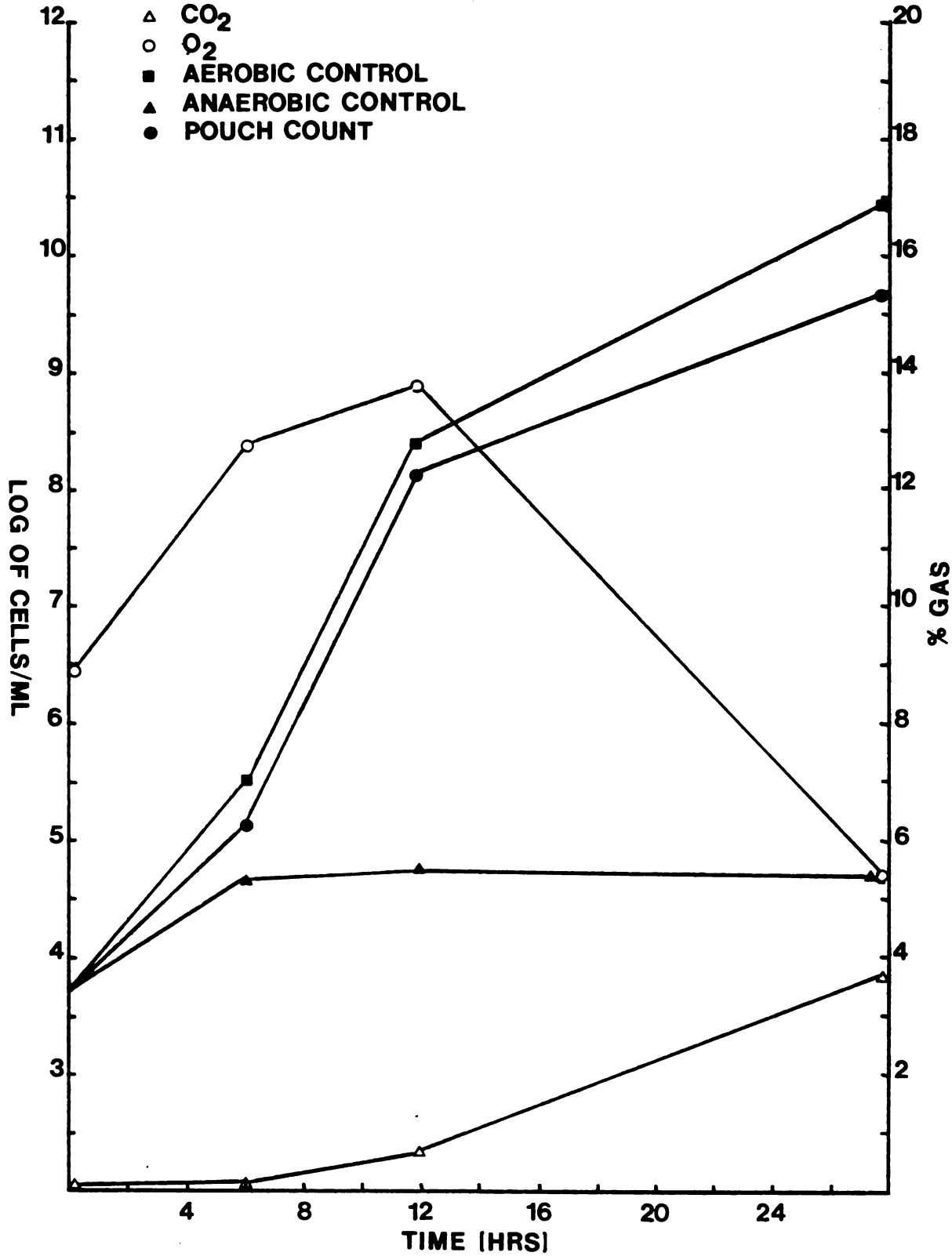


FIGURE 4 - POLYETHYLENE GROWTH CURVE

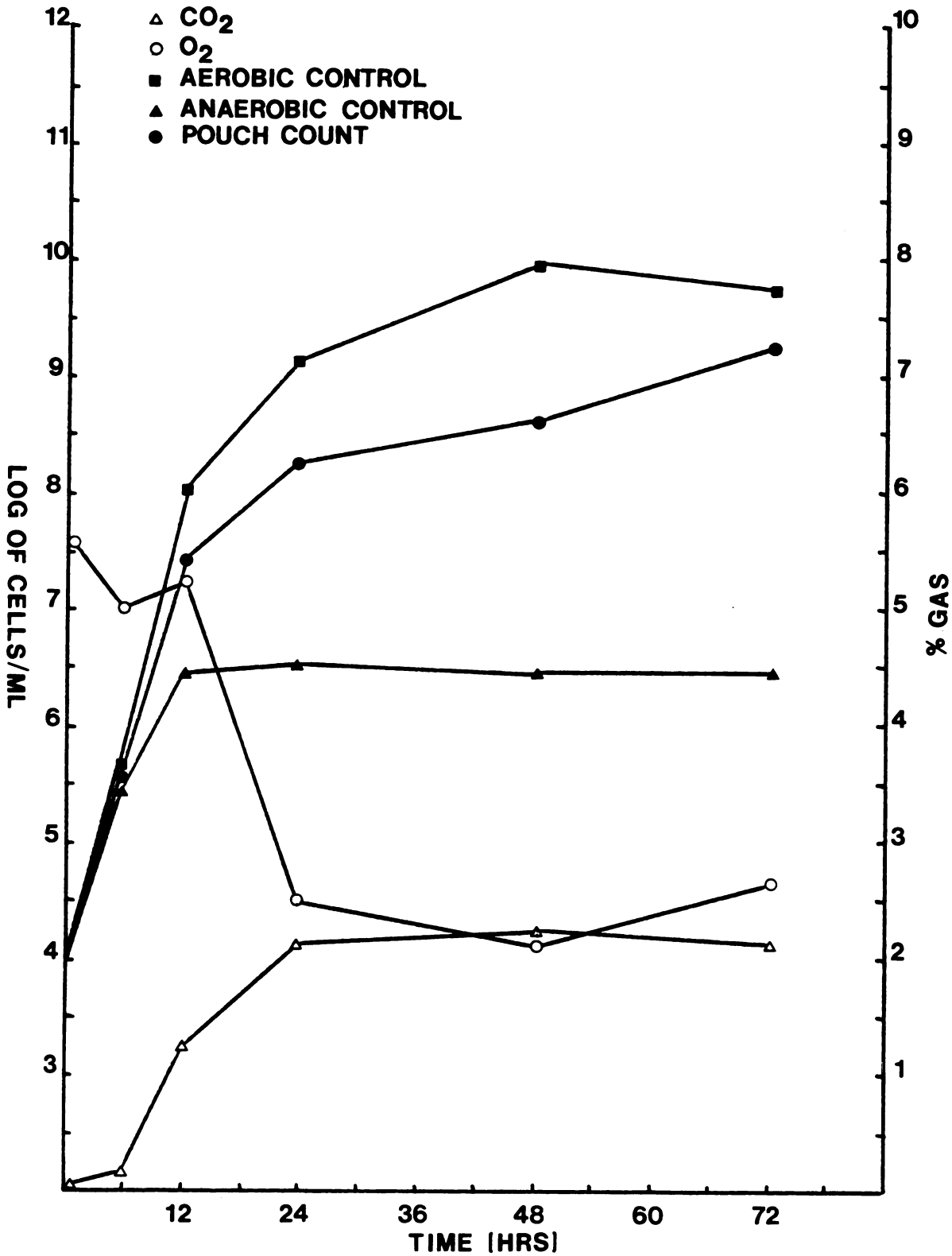


FIGURE 5 - SARAN COATED NYLON GROWTH CURVE

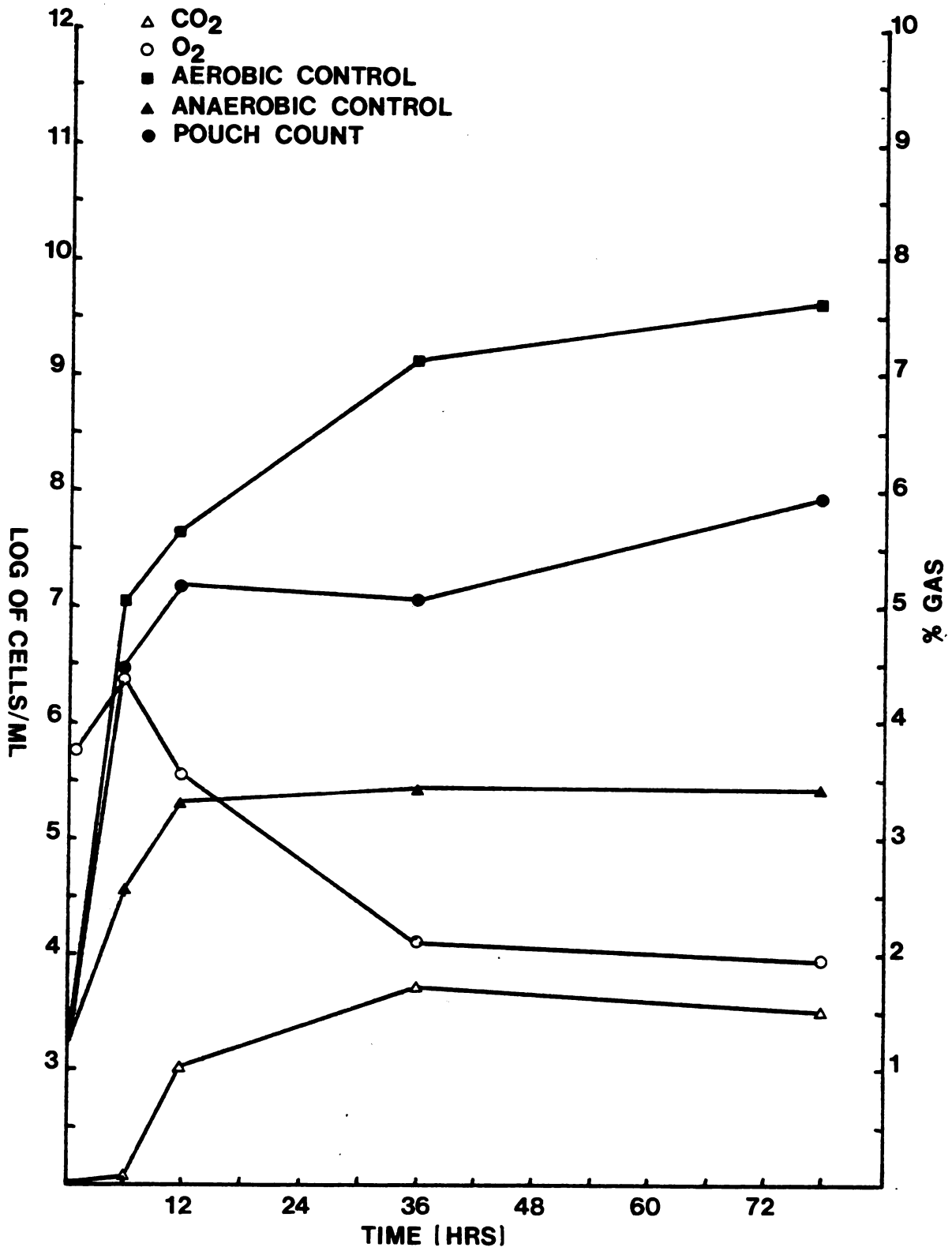


FIGURE 6 - SARAN GROWTH CURVE

inhibited, with respect to total cell yield, in these films than in the polyethylene.

To better quantify the degree of inhibition the culture is experiencing, calculations are made to determine what percentage the bag's colony count is of the aerobic control's colony count. The aerobic control's colony count is used as 100% and is calculated at the same time point for each film.

$$\% \text{ of Aerobic Control} = \frac{\text{Bag colony count}}{\text{Aerobic Control Colony Count}} \times 100\%$$

The higher the percentage of Aerobic control, the less the inhibition and the lower the percentage, the greater the inhibition. Table 5 shows the results of these films calculated at 24 hours.

Table 5
Growth Inhibition

Film	Bag Colony Count (cells/ml)	Aerobic Control Colony Count (cells/ml)	% of Aerobic Control
PE	2.00×10^9	8.91×10^9	22.4
SN	1.90×10^8	1.55×10^9	12.2
S	1.26×107	2.51×10^8	5.0

The percent of Aerobic Control figure in Table 5 support the contention that inhibition increases with decreasing gas permeability of the film used. It should be noted that it appears that this inhibition may increase even further later in the growth cycle, although this cannot be calculated, due to a lack of colony counts for polyethylene past 28 hours.

It is not clear whether the inhibition is due to O_2 depletion or CO_2 increase. It is most likely a combination of both. Apparently, since the O_2 level in the headspace drops, some O_2 in the atmosphere is being used up by the organism. More specifically, the bacteria is utilizing the O_2 in solution and more is taking it's place from the headspace. The fact that all samples have a similar growth rate up to 12 hours is probably due to the culture utilizing O_2 left in solution.

If one mole of CO_2 is produced for every mole of O_2 in the metabolism of Pseudomonas, through the amount of O_2 consumed should equal the amount of CO_2 produced. Oxygen consumption can be described by the following equation.

$$\text{Total } O_2 \text{ Consumed} = \begin{array}{l}\text{Initial Amount (headspace)} \\ + \text{ Amount Permeated In} \\ + \text{ Amount in Solution Initially} \\ - \text{ Amount In Solution (Final)} \\ - \text{ Final Concentration (headspace)}\end{array}$$

$$\text{Initial Amount} = \%\ O_2 \text{ initially} \times 20 \text{ cc headspace volume}$$

$$\text{Amount Permeated In} = \text{Permeability Rate} \times \text{Time} \times \text{Driving Force} \times \text{Surface Area}$$

$$\text{Amount In Solution Initially} = 0.02541\ ^{cc}/ml \times 20 \text{ ml (broth)} \times \%\ O_2 \text{ initial}$$

$$\text{Amount in Solution Final} = 0.02541\ ^{cc}/ml \times 20 \text{ ml (broth)} \times \%\ O_2 \text{ final}$$

$$\text{Final Concentration} = \%\ O_2 \text{ final} \times 20 \text{ cc headspace volume}$$

Note: The driving force used in the amount permeated in calculation is taken from the point where the O_2 concentration starts to level off for the saran and saran coated nylon samples and half way between the highest and lowest O_2

concentration for polyethylene. The calculation of the O_2 solubility constants are given in Appendix D.

CO_2 production is described in the following equation.

Total CO_2 Produced = Amount In Headspace Final + Amount In Solution (highest level) + Amount Permeated Out

- Atmospheric Level of CO_2

Amount in Headspace Final = % CO_2 final x 20 cc headspace volume

Amount in Solution = 0.650 $^{cc}/ml$ x 20 ml (broth) x % CO_2 (highest level)

Amount Permeated Out = Permeability x Time x Driving Rate (days) Force x Surface Area

Atmospheric CO_2 Level = 0.033% x 20 cc headspace volume

Note: The driving force used for the amount permeated out calculation is the average of the lowest and highest CO_2 levels. The surface area is, 8 in. x 6 in. = 48 $in.^2$ (one side)
48 $in.^2$ x 0.000645 m^2/in^2 = 0.03 m^2 x 2 sides = 0.06 m^2
Also, the value for the atmospheric level of CO^2 was obtained from the "Handbook of Chemistry and Physics," 53rd edition.

Total O_2 consumption and CO_2 production calculation results are shown in Tables 6 and 7, respectively.

Table 6
O_2 Consumption

Film	+A	+B	+C	-D	-E	Total O_2 Consumption (cc)
PE	1.8	39.7	0.04	0.03	1.1	40.4
SN	1.1	0.5	0.03	0.01	0.5	1.1
S	0.7	0.5	0.02	0.01	0.4	0.8

Note: A = Initial Amount (cc)
B = Amount Permeated In (cc)

C = Amount In Solution Initially (cc)
D = Amount In Solution Final (cc)
E = Final concentration (cc)

Table 7
CO_2 Production

Film	+F	+G	+H	-I	Total CO_2 Production (cc)
PE	0.8	0.49	55.8	0.01	57.1
SN	0.4	0.35	0.3	0.01	1.0
S	0.3	0.20	0.2	0.01	0.7

Note: F = Amount In Headspace Final (cc)
G = Amount In Solution (cc)
H = Amount Permeated Out (cc)
I = Atmospheric CO_2 Level (cc)

The values calculated for O_2 consumption and CO_2 production match quite well, except for polyethylene. In that sample O_2 consumption is considerably lower than CO_2 production. This may be due to an error in the amount permeated in calculation. If the equilibrium O_2 concentration is used in calculating the driving force, B now equals 54.5 cc and the Total O_2 Consumption equals 55.2 cc, which is much closer to the CO_2 production value.

The fact that O_2 consumption and CO_2 production are approximately equal indicate that the change in the concentration of headspace gases are mostly or entirely due to the metabolism of Pseudomonas aeruginosa and permeability rates of the films. If this was not true the CO_2 production would not equal the amount of O_2 consumed.

The results in Tables 6 and 7 suggest that the organisms are consuming approximately 50 times as much O_2 in the PE than in the higher barrier film. This conclusion is

not confirmed by the colony count measurements, since they do not show a fifty-fold increase in population. It must be remembered that respiratory rates of bacteria are not constant throughout it's growth. Also, cell concentrations and O_2 headspace concentrations were not exactly the same at the outset of the run. Combine this with the fact that the respiratory quotients change, through the growth cycle of the microbe, one would doubt there is a linear relationship between O_2 consumption and cell concentration over the length of the run.

Earlier the statement was made that inhibition of total cell yield was evident in the stationary phase. For this to be true, and to prove that the permeability of the film governs total cell yield, and analysis of the quantity of O_2 permeating in versus the amount of O_2 being consumed by bacterial metabolism must be made. Research on Pseudomonas fluorescens showed that it's respiratory rate increased during exponential phase and decreased, tending toward a value of 3 cc O_2/g dry wt./min. during stationary phase (Sadoff et al, 1956). To be able to use the value in conjunction with the data collected in this research one must know the weight of an individual cell and it's percent water by weight. According to (Nester, 1973) one bacterium weighs 5.0×10^{-12} g wet wt./cell and is 70% water by weight. Therefore,

$$1 \text{ bacterium} = 5.0 \times 10^{-12} \text{ g wet wt./cell} \times 0.30$$
$$= 1.5 \times 10^{-12} \text{ g dry wt./cell.}$$

With this information the analysis can be made. First, the grams in dry weight of cells at time t must be determined using the following set of equations.

(cell conc. at time t (cells/ml)) x 20 ml (broth) = no. of cells

(No. of cells) x (1.5×10^{-12} g dry wt./cell) = dry cells (g)

From this the amount of O_2 being consumed by bacterial metabolism at time t can be calculated.

(3 cc O_2/g dry wt./min) x (grams of dry cells) = Amount O_2 being consumed at time t (cc/min)

This value can then be compared with the amount of O_2 permeating into the package, which is calculated in the following manner.

$$\text{Permeability Rate of Film } \left(\frac{cc\,O_2}{m^2\ day\ atm}\right) \times \frac{1\ day}{1440\ min} \times \text{Driving Force at Time t} \times \text{Surface Area}$$

= Amount O_2 permeating in at time t (cc/min.)

The results of these calculations are shown in Table 8.

Table 8
O_2 Availability and Consumption

Film	A	B	C	D	Amount O_2 Consumed (cc/min)	Amount O_2 Permeated In (cc/min)
PE	24	2×10^{9}	4×10^{10}	0.060	0.180	0.02800
SN	60	8.9×10^{6}	1.8×10^{8}	0.00027	0.00081	0.00013
S	60	3.55×10^{7}	7.1×10^{8}	0.00106	0.0032	0.0001

Note: A = Time (hrs.)
B = Cell Concentration at Time t (cells/ml)
C = No. of Cells
D = Dry Cells (g)

For each film the amount of O_2 permeating in is less

than the amount required by the culture. Therefore, the O_2 permeability of the film must be exerting, to some degree, an inhibitory effect on the growth of the bacteria. In the graphs (Figures 4, 5 and 6) this inhibitory effect appears to be manifested in a decrease in total cell yield at time t and it's magnitude is related to the O_2 permeability of the film. Also, since CO_2 has been shown to be inhibitory to Pseudomonas, it may be more correct to speak of O_2 being a limiting factor to bacterial growth at a certain CO_2 concentration. Both the O_2 and CO_2 concentrations are in part determined by the permeability of the film being used and in part by bacterial metabolism.

Although the values calculated in Tables 6 and 7 for O_2 consumption and CO_2 production are approximately equal, there is a slight discrepancy. This might be explained by looking at an isolated section of the graph; the area where the headspace gases come to equilibrium. At this phase the respiratory rate starts to level off and becomes more constant (Sadoff, 1956), causing the gases to have a more constant concentration. Now, with a constant respiratory rate, the amount of dissolved O_2 and CO_2 in solution becomes constant and the amount of O_2 and CO_2 in the headspace reaches equilibrium. With those assumptions made, the respiratory quotient can be described by the amount of O_2 permeating in the pouch and the amount of CO_2 permeating out. Figure 7 graphically illustrates this concept.

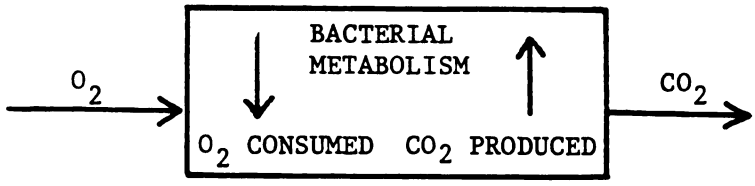


Figure 7

Pouch Gas Dynamics

By calculating the permeability rates of the films to O_2 and CO_2, the respiratory quotient can be determined.

O_2 Permeability Rate at Equilibrium (cc/day) = Permeability Rate at 1 atm (cc/m^2 day atm) x Surface Area (m^2) x Driving Force (atm)

CO_2 Permeability Rate at Equilibrium (cc/day) = Permeability Rate at 1 atm (cc/m^2 day atm) x Surface Area (m^2) x Driving Force (atm)

The results of these calculations are summarized in Table 9.

Table 9
Respiratory Quotients (Stationary Phase)

Film	O_2 Permeability at Equilibrium (cc/day)	CO_2 Permeability at Equilibrium (cc/day)	Respiratory Quotient CO_2:O_2
PE	49.20	57.18	1:1.16
SN	0.19	0.09	1:2.11
S	0.14	0.06	1:2.33

The respiratory quotients tend to increase, with respect to O_2 demand, in the higher barrier film, but not significantly in the lower barrier film. Therefore, the change in respiratory quotients in the high barrier films is most likely due to the organism being stressed by the combination of O_2 reduction and CO_2 increase in the

headspace. The respiratory quotient of the bacteria in the polyethylene bag did not change significantly, because the organisms were not as stressed as the ones in the high barrier films. This is evident by recalling that the colony counts for the polyethylene bags were only slightly less than the aerobic control, which shows a lesser degree of stress on the cells.

To confirm these observations it is necessary to go back and calculate the respiratory quotients of the bacteria during it's exponential growth phase. The 0-12 hour interval was chosen for these calculations, because it best represents the exponential phase of growth. The same equations are used to determine O_2 consumption and CO_2 production during this time interval, as was used previously in Tables 6 and 7. The results of these calculations are displayed in Tables 10 and 11.

Table 10
Total O_2 Consumed

Film	A	+	B	+	C	-	D	-	E	=	Total O_2 Consumed (cc)
PE	1.78		10.7		0.04		0.07		2.76		9.69
SN	1.11		0.32		0.03		0.03		1.05		0.38
S	0.75		0.29		0.02		0.02		0.71		0.33

Table 11
Total CO_2 Produced

Film	F	+	G	+	H	-	I	=	Total CO_2 Produced (cc)
PE	0.14		0.09		6.20		0.01		6.42
SN	0.25		0.16		0.02		0.01		0.42
S	0.20		0.13		0.02		0.01		0.34

The respiratory quotients can now be calculated for each film over this time period, by comparing the amount of O_2 consumed to this amount of CO_2 produced. These comparisions are shown in Table 12.

Table 12
Respiratory Quotients (Exponential Phase)

Film	Respiratory Quotients (CO_2:O_2)
PE	0.66:1
SN	1.10:1
S	1.03:1

From the table it is evident that the saran and saran coated nylon pouches adhere very well to the predicted 1:1 respiratory quotient. The polyethylene sample seemed to have strayed more from the 1:1 quotient. This is most likely due to the amount of accuracy in the CO_2 headspace measurement, since a small amount of error would cause a large change in the respiratory quotient.

Therefore, it has been observed that Pseudomonas aeruginosa follows typical metabolic processes at first, until the majority of the O_2 dissolved in the media is used up. Once this is gone, and providing the bacteria is enclosed in a barrier material it's metabolic processes appear to change, apparently from the stresses caused by a increased CO_2 concentration. This concept was not evident in the low gas barrier material, which indicates that permeability plays an important role in determining the metabolic pathways used by the organism. This stress is also evidenced by the reduction in cell yield, as previously

discussed.

SUMMARY AND CONCLUSIONS

The permeable pouch is a very dynamic system with respect to microbiological growth and headspace gases. Several observations were made with respect to this dynamic process.

It was found that the number of colony forming units decreases with decreasing permeability of the film used.

There was a reduction in O_2 concentration in the headspace and an increase in CO_2 concentration as the number of organisms increased. Also, the headspace gases tended to an equilibrium, once the bacteria reached stationary phase, where the O_2 and CO_2 concentrations are equal.

The O_2 and CO_2 concentrations are determined by the permeability of the film and the bacterial metabolism.

O_2 consumption and CO_2 production by the bacteria can be calculated by considering the permeability of the film, dissolved gases in solution and gas concentrations in the headspace.

The respiratory quotient of the organisms can be calculated by comparing O_2 and CO_2 headspace concentrations at the equilibrium phase.

Placing bacteria in a barrier film causes stresses on the microbes, by reducing the concentration of O_2 and increasing the concentration of CO_2 in the headspace. This stress appears to cause a change in the organism's metabolism, thus changing it's respiratory quotient.

RECOMMENDATIONS

The research done here is enough to show that a pattern exists between microbial growth, film permeability and headspace gases. This opens up some intriguing possibilities, such as computer prediction of cell yield given the permeability of the film, initial headspace has concentrations and initial cell concentration. A concept such as this could greatly aid shelf-life predictions of many food products that are packaged in gas permeable packages.

Before computerized shelf-life predictions can become a reality much work needs to be done. Better precision in the quantification of data is necessary. More precise measurements of headspace gases and a quicker method of cell counts must be devised, since time is an important consideration. A method of equilibrating the amount of dissolved O_2 in the media before inoculation of the organisms is needed. A great quantity of data is needed for a data base. Finally, this experiment does not consider the mixed flora effects. Many contaminated food products contain more than one organism, which could lead to neighbor-neighbor inhibition and microbial succession, which would probably alter the results obtained here.

APPENDIX

Appendix A

Headspace Measurements

Appendix A

Headspace Measurements

Table A1

Polyethylene (2 mil)

Time (hrs)	Pouch	Gas	Peak Height	Peak Width	Peak Area	AU/%	Gas % v/v
0	CAL	O_2	7.500	0.1250	0.938	0.062	--
0	CAL	CO_2	2.700	0.100	0.270	0.054	--
0	PE 1	O_2	5.350	0.100	0.540	--	8.56
0	PE 1	CO_2	0	0	0	--	0
0	PE 2	O_2	6.100	0.100	0.610	--	9.76
0	PE 2	CO_2	0	0	0	--	0
0	PE 3	O_2	5.400	0.100	0.540	--	8.64
0	PE 3	CO_2	0	0	0	--	0
6	CAL	O_2	7.500	0.100	0.750	0.050	--
6	CAL	CO_2	2.500	0.120	0.300	0.062	--
6	PE 1	O_2	6.350	0.100	0.635	--	12.7
6	PE 1	CO_2	0.075	0.075	0.006	--	0.018
6	PE 2	O_2	6.500	0.100	0.650	--	13.0
6	PE 2	CO_2	0	0	0	--	0
6	PE 3	O_2	6.300	0.100	0.630	--	12.6
6	PE 3	CO_2	0	0	0	--	0
12	CAL	O_2	7.450	0.100	0.745	0.050	--
12	CAL	CO_2	2.700	0.125	0.338	0.068	--
12	PE 1	O_2	7.150	0.100	0.715	--	14.39
12	PE 1	CO_2	0.600	0.100	0.060	--	0.89
12	PE 2	O_2	6.800	0.100	0.680	--	13.68
12	PE 2	CO_2	0.300	0.100	0.030	--	0.44
12	PE 3	O_2	6.650	0.100	0.665	--	13.38
12	PE 3	CO_2	0.400	0.100	0.040	--	0.59
28	CAL	O_2	7.550	0.100	0.755	0.050	--
28	CAL	CO_2	2.500	0.100	0.250	0.050	--
28	PE 1	O_2	2.850	0.100	0.285	--	5.67
28	PE 1	CO_2	1.900	0.100	0.190	--	3.80
28	PE 2	O_2	3.550	0.100	0.355	--	7.06
28	PE 2	CO_2	1.600	0.100	0.160	--	3.20
28	PE 3	O_2	1.650	0.100	0.165	--	3.28
28	PE 3	CO_2	2.150	0.100	0.215	--	4.30

Table A?

Saran Coated Nylon (.75 mil)

Time (hrs)	Pouch	Gas	Peak Height	Peak Width	Peak Area	AU/%	Gas % v/v
0	CAL	O_2	6.100	0.125	0.762	0.051	--
0	CAL	CO_2	2.650	0.150	0.398	0.080	--
0	SN 1	O_2	3.000	0.100	0.300	--	5.88
0	SN 1	CO_2	0	0	0	--	0
0	SN 2	O_2	2.750	0.100	0.275	--	5.39
0	SN 2	CO_2	0	0	0	--	0
0	SN 3	O_2	2.800	0.100	0.280	--	5.49
0	SN 3	CO_2	0	0	0	--	0
6	CAL	O_2	6.000	0.100	0.600	0.040	--
6	CAL	CO_2	2.600	0.150	0.390	0.078	--
6	SN 1	O_2	2.100	0.100	0.210	--	5.25
6	SN 1	CO_2	0.100	0.100	0.010	--	0.13
6	SN 2	O_2	2.000	0.100	0.200	--	5.00
6	SN 2	CO_2	0.150	0.100	0.015	--	0.19
6	SN 3	O_2	1.900	0.100	0.190	--	4.75
6	SN 3	CO_2	0.150	0.100	0.015	--	0.19
12	CAL	O_2	6.000	0.100	0.600	0.040	--
12	CAL	CO_2	2.600	0.125	0.325	0.065	--
12	SN 1	O_2	2.100	0.100	0.210	--	5.25
12	SN 1	CO_2	0.600	0.125	0.075	--	1.15
12	SN 2	O_2	2.000	0.100	0.200	--	5.00
12	SN 2	CO_2	0.600	0.125	0.075	--	1.15
12	SN 3	O_2	2.200	0.100	0.220	--	5.50
12	SN 3	CO_2	0.800	0.125	0.100	--	1.54
24	CAL	O_2	6.000	0.100	0.600	0.040	--
24	CAL	CO_2	2.600	0.125	0.325	0.065	--
24	SN 1	O_2	0.900	0.100	0.090	--	2.25
24	SN 1	CO_2	1.090	0.125	0.136	--	2.10
24	SN 2	O_2	1.100	0.100	0.110	--	2.75
24	SN 2	CO_2	1.140	0.125	0.143	--	2.20
24	SN 3	O_2	1.000	0.100	0.100	--	2.50
24	SN 3	CO_2	1.040	0.125	0.130	--	2.00
48	CAL	O_2	5.700	0.100	0.570	0.038	--
48	CAL	CO_2	2.600	0.125	0.325	0.065	--
48	SN 1	O_2	0.850	0.100	0.085	--	2.24
48	SN 1	CO_2	1.09	0.125	0.136	--	2.10
48	SN 2	O_2	0.800	0.100	0.080	--	2.10
48	SN 2	CO_2	1.170	0.125	0.146	--	2.25
48	SN 3	O_2	0.750	0.100	0.075	--	1.97
48	SN 3	CO_2	1.270	0.125	0.159	--	2.45

Table A2 (cont'd.)

Time (hrs)	Pouch	Gas	Peak Height	Peak Width	Peak Area	AU/%	Gas % v/v
72	CAL	O_2	5.400	0.100	0.540	0.036	--
72	CAL	CO_2	2.500	0.125	0.312	0.062	--
72	SN 1	O_2	1.200	0.100	0.120	--	3.33
72	SN 1	CO_2	0.975	0.125	0.121	--	1.95
72	SN 2	O_2	0.800	0.100	0.080	--	2.22
72	SN 2	CO_2	1.090	0.125	0.136	--	2.20
72	SN 3	O_2	0.900	0.100	0.090	--	2.50
72	SN 3	CO_2	1.120	0.125	0.140	--	2.25

Table A3

Saran (2 mil)

Time (hrs)	Pouch	Gas	Peak Height	Peak Width	Peak Area	AU/%	Gas % v/v
0	CAL	O_2	7.200	0.100	0.720	0.048	--
0	CAL	CO_2	3.600	0.100	0.36	0.072	--
0	S 1	O_2	2.500	0.075	0.188	--	3.92
0	S 1	CO_2	0	0	0	--	0
0	S 2	O_2	1.750	0.100	0.175	--	3.64
0	S 2	CO_2	0	0	0	--	0
0	S 3	O_2	1.750	0.100	0.175	--	3.64
0	S 3	CO_2	0	0	0	--	0
6	CAL	O_2	7.600	0.100	0.760	0.051	--
6	CAL	CO_2	2.800	0.100	0.280	0.056	--
6	S 1	O_2	2.600	0.100	0.260	--	5.10
6	S 1	CO_2	0	0	0	--	0
6	S 2	O_2	2.150	0.100	0.215	--	4.22
6	S 2	CO_2	0	0	0	--	0
6	S 3	O_2	1.950	0.100	0.195	--	3.82
6	S 3	CO_2	0	0	0	-	0
12	CAL	O_2	7.600	0.100	0.760	0.051	--
12	CAL	CO_2	2.800	0.100	0.280	0.056	--
12	S 1	O_2	1.950	0.100	0.195	--	3.82
12	S 1	CO_2	0.750	0.075	0.056	--	1.00
12	S 2	O_2	1.850	0.100	0.185	--	3.63
12	S 2	CO_2	0.750	0.075	0.056	--	1.00
12	S 3	O_2	1.650	0.100	0.165	--	3.24
12	S 3	CO_2	0.750	0.075	0.56	--	1.00
36	CAL	O_2	7.300	0.100	0.730	0.049	--
36	CAL	CO_2	2.200	0.100	0.220	0.044	--
36	S 1	O_2	1.200	0.100	0.120	--	2.44
36	S 1	CO_2	0.750	0.100	0.075	--	1.70
36	S 2	O_2	0.950	0.100	0.95	--	1.94
36	S 2	CO_2	0.700	0.100	0.070	--	1.43
36	S 3	O_2	1.000	0.100	0.100	--	2.04
36	S 3	CO_2	0.550	0.125	0.069	--	1.57
78	CAL	O_2	7.700	0.100	0.770	0.051	--
78	CAL	CO_2	2.550	0.100	0.255	0.51	--
78	S 1	O_2	1.100	0.100	0.110	--	2.16
78	S 1	CO_2	0.750	0.100	0.75	--	1.47
78	S 2	O_2	1.000	0.100	0.100	--	1.96
78	S 2	CO_2	0.800	0.100	0.080	--	1.57
78	S 3	O_2	0.900	0.100	0.090	--	1.76
78	S 3	CO_2	0.700	0.100	0.070	--	1.37

Table A4

Polyethylene (2 mil)

Time	Gas	$\overline{\%\ v/v}$	Std. Dev.
0	O_2	8.99	0.67
0	CO_2	0	0
6	O_2	12.77	0.21
6	CO_2	0.01	0.01
12	O_2	13.82	0.52
12	CO_2	0.64	0.23
28	O_2	5.34	1.91
28	CO_2	3.77	0.55

Table A5

Saran Coated Nylon (.75 mil)

Time	Gas	$\overline{\%\ v/v}$	Std. Dev.
0	O_2	5.59	0.26
0	CO_2	0	0
6	O_2	5.00	0.25
6	CO_2	0.17	0.03
12	O_2	5.25	0.25
12	CO_2	1.28	0.22
24	O_2	2.50	0.25
24	CO_2	2.10	0.10
48	O_2	2.10	0.14
48	CO_2	2.27	0.18
72	O_2	2.68	0.58
72	CO_2	2.13	0.16

Table A6

Saran (2 mil)

Time	Gas	% v/v	Std. Dev.
0	O_2	3.73	0.16
0	CO_2	0	0
6	O_2	4.38	0.65
6	CO_2	0	0
12	O_2	3.56	0.30
12	CO_2	1.00	0
36	O_2	2.14	0.26
36	CO_2	1.57	0.14
78	O_2	1.96	0.20
78	CO_2	1.47	0.10

All peak widths were measured at 1/2 the peak height.

$$\text{Peak Area} = \text{Peak Height} \times \text{Peak Width}$$

The gas chromatograph was calibrated for oxygen and carbon dioxide at each time interval. The peak area calculated represents the response for a sample of 15% O_2 and 5% CO_2. These values must be converted to Area units/% gas, so they may be used to calculate headspace gas concentrations. Therefore,

$$O_2 \text{ calibration} = \frac{\text{Peak Area}}{15\%} = \frac{\text{Area Units}}{\% \text{ gas}}$$

and

$$CO_2 \text{ calibration} = \frac{\text{Peak Area}}{5\%} = \frac{\text{Area Units}}{\% \text{ gas}}$$

To calculate the concentration of gases in the headspace the Peak Area for the respective gas is divided by the calibration factor for that gas.

$$\% \text{ gas (volume/volume)} = \frac{\text{Peak Area}}{\text{Area Units}/\%}$$

Appendix B

Colony Counts

Appendix B

Colony Counts

Table B1

Polyethylene (2 mil)

Time (hrs.)	Pouch	Colony Count	Log of Colony Count	$\overline{\text{Log of Colony Count}}$	Std. Dev.
0	All	5.65×10^3	3.75	--	--
0	All	4.75×10^3	3.68	3.71	0.05
6	AER	4.10×10^5	5.61	--	--
6	AER	2.50×10^5	5.40	5.50	0.15
6	ANA	4.90×10^4	4.69	--	--
6	ANA	4.10×10^4	4.61	4.65	0.06
6	PE 1	1.55×10^5	5.19	--	--
6	PE 1	1.29×10^5	5.11	5.15	0.06
6	PE 2	1.46×10^5	5.16	--	--
6	PE 2	1.28×10^5	5.11	5.14	0.04
6	PE 3	1.52×10^5	5.18	--	--
6	PE 3	1.46×10^5	5.16	5.17	0.01
12	AER	2.57×10^8	8.41	--	--
12	AER	2.49×10^8	8.40	8.40	0.01
12	ANA	5.50×10^4	4.74	--	--
12	ANA	5.10×10^4	4.71	--	--
12	PE 1	1.30×10^8	8.11	--	--
12	PE 1	1.24×10^8	8.09	8.10	0.01
12	PE 2	1.37×10^8	8.14	--	--
12	PE 2	1.21×10^8	8.08	8.11	0.04
12	PE 3	1.74×10^8	8.24	--	--
12	PE 3	1.66×10^8	8.22	8.23	0.01
28	AER	2.86×10^{10}	10.45	--	-
28	AER	2.64×10^{10}	10.42	10.44	0.02
28	ANA	5.8×10^4	4.76	--	--
28	ANA	5.2×10^4	4.72	4.74	0.03
28	PE 1	4.8×10^9	9.68	--	--
28	PE 1	3.6×10^9	9.56	9.62	0.08
28	PE 2	5.9×10^9	9.77	--	--
28	PE 2	4.7×10^9	9.67	9.72	0.07
28	PE 3	4.7×10^9	9.67	--	--
28	PE 3	4.5×10^9	9.65	9.66	0.01

Table B2

Saran (2 mil)

Time (hrs)	Pouch	Colony Count	Log of Colony Count	$\overline{\text{Log of Colony Count}}$	Std. Dev.
0	All	1.76×10^3	3.24	--	--
0	All	1.54×10^3	3.19	3.22	0.04
6	AER	1.09×10^7	7.04	--	--
6	AER	1.01×10^7	7.00	7.02	0.03
6	ANA	4.00×10^4	4.60	--	--
6	ANA	2.80×10^4	4.45	4.52	0.11
6	S 1	2.80×10^6	6.45	--	--
6	S 1	2.68×10^6	6.43	6.44	0.01
6	S 2	2.86×10^6	6.46	--	--
6	S 2	2.80×10^6	6.45	6.46	0.01
6	S 3	2.98×10^6	6.47	--	--
6	S 3	2.84×10^6	6.45	6.46	0.01
12	AER	4.90×10^7	7.69	--	--
12	AER	4.10×10^7	7.61	7.65	0.06
12	ANA	1.68×10^5	5.22	--	--
12	ANA	1.54×10^5	5.19	5.20	0.02
12	S 1	1.55×10^7	7.19	--	--
12	S 1	1.51×10^7	7.18	7.18	0.01
12	S 2	1.13×10^7	7.05	--	--
12	S 2	1.03×10^7	7.01	7.03	0.03
12	S 3	1.35×10^7	7.13	--	--
12	S 3	1.27×10^7	7.10	7.12	0.02
36	AER	1.37×10^9	9.14	--	--
36	AER	1.27×10^9	9.10	9.12	0.03
36	ANA	2.56×10^5	5.41	--	--
36	ANA	2.50×10^5	5.40	5.40	0.01
36	S 1	9.90×10^6	7.00	--	--
36	S 1	9.50×10^6	6.98	6.99	0.01
36	S 2	1.39×10^7	7.14	--	--
36	S 2	1.31×10^7	7.12	7.13	0.01
36	S 3	1.03×10^7	7.01	--	--
36	S 3	9.10×10^6	6.96	6.98	0.04
78	AER	4.50×10^9	9.65	--	--
78	AER	4.10×10^9	9.61	9.63	0.03
78	ANA	2.56×10^5	5.41	--	--
78	ANA	2.50×10^5	5.40	5.40	0.01
78	S 1	1.00×10^8	8.00	--	--
78	S 1	8.80×10^7	7.94	7.97	0.04
78	S 2	9.00×10^7	7.95	--	--
78	S 2	8.20×10^7	7.91	7.93	0.03
78	S 3	8.60×10^7	7.93	--	--
78	S 3	7.60×10^7	7.88	7.90	0.04

Table B3

Saran coated nylon (0.75 mil)

Time (hrs)	Pouch	Colony Count	Log of Colony Count	$\overline{\text{Log of Colony Count}}$	Std. Dev.
0	All	9.48×10^3	3.98	--	--
0	All	9.42×10^3	3.97	3.98	0.01
6	AER	3.70×10^5	5.57	--	--
6	AER	2.70×10^5	5.43	5.50	0.10
6	ANA	3.30×10^5	5.52	--	--
6	ANA	2.90×10^5	5.46	5.49	0.04
6	SN 1	5.50×10^5	5.74	--	--
6	SN 1	4.30×10^5	5.63	5.68	0.08
6	SN 2	4.10×10^5	5.61	--	--
6	SN 2	3.30×10^5	5.52	5.56	0.06
6	SN 3	4.60×10^5	5.66	--	--
6	SN 3	4.00×10^5	5.60	5.63	0.04
12	AER	1.11×10^8	8.04	--	--
12	AER	9.70×10^7	7.99	8.01	0.04
12	ANA	2.94×10^6	6.47	--	--
12	ANA	2.78×10^6	6.44	6.46	0.02
12	SN 1	3.04×10^7	7.48	--	--
12	SN 1	2.86×10^7	7.46	7.47	0.01
12	SN 2	2.96×10^7	7.47	--	--
12	SN 2	2.70×10^7	7.43	7.45	0.03
12	SN 3	2.86×10^7	7.46	--	--
12	SN 3	2.66×10^7	7.42	7.44	0.03

Table B3 (cont'd.)

Time (hrs.)	Pouch	Colony Count	Log of Colony Count	$\overline{\text{Log of Colony Count}}$	Std. Dev.
24	AER	1.61×10^9	9.21	--	--
24	AER	1.49×10^9	9.17	9.19	0.03
24	ANA	3.00×10^6	6.48	--	--
24	ANA	2.88×10^6	6.46	6.47	0.01
24	SN 1	2.07×10^8	8.32	--	--
24	SN 1	1.91×10^8	8.28	8.30	0.03
24	SN 2	1.90×10^8	8.28	--	--
24	SN 2	1.66×10^8	8.22	8.25	0.04
24	SN 3	2.05×10^8	8.31	--	--
24	SN 3	2.01×10^8	8.30	8.30	0.08
48	AER	1.00×10^{10}	10.00	--	--
48	AER	9.20×10^9	9.96	9.98	0.03
48	ANA	3.06×10^6	6.48	--	--
48	ANA	2.90×10^6	6.46	6.47	0.01
48	SN 1	4.60×10^8	8.66	--	--
48	SN 1	2.40×10^8	8.38	8.52	0.20
48	SN 2	4.70×10^8	8.67	--	--
48	SN 2	3.70×10^8	8.57	8.62	0.07
48	SN 3	4.90×10^8	8.69	--	--
48	SN 3	4.10×10^8	8.61	8.65	0.06
72	AER	6.30×10^9	9.80	--	--
72	AER	4.90×10^9	9.69	9.74	0.08
72	ANA	3.08×10^6	6.49	--	--
72	ANA	2.80×10^6	6.45	6.47	0.03
72	SN 1	1.82×10^9	9.26	--	--
72	SN 1	1.66×10^9	9.22	9.24	0.03
72	SN 2	1.92×10^9	9.28	--	--
72	SN 2	1.80×10^9	9.26	9.27	0.01
72	SN 3	1.76×10^9	9.24	--	--
72	SN 3	1.62×10^9	9.21	9.22	0.02

Table B4

Polyethylene (2 mil)

Time	Log of Colony Count of Pouches	Std. Dev.
6	5.15	0.02
12	8.15	0.07
28	9.67	0.05

Table B5

Saran (2 mil)

Time	Log of Colony Count of Pouches	Std. Dev.
6	6.45	0.01
12	7.11	0.08
24	7.03	0.08
78	7.93	0.04

Table B6

Saran coated nylon (0.75 mil)

Time	Log of Colony Count of Pouches	Std. Dev.
6	5.62	0.06
12	7.45	0.02
24	8.28	0.03
48	8.60	0.07
72	9.24	0.02

Appendix C

Film Permeability Measurements

Appendix C

Film Permeability Measurements

Oxygen (Oxtran 100)

The oxygen permeability of the films was calculated by multiplying the calibration factor (determined by the resistor used) by the mV response observed on the chart recorder.

$$O_2 \text{ permeability} = \text{mV response} \times \frac{(X) \text{ cc/m2/24 hrs.}}{1 \text{ mV}}$$

Polyethylene (2 mil)

sensitivity - 100 mV full scale

5.3 Ohm resistor: $\frac{100 \text{ cc/m}^2\text{/24 hrs.}}{\text{mV}}$

Runs made at 32°C and 0% RH

Table C1
Polyethylene mV Response

Run No.	mV Response
1	50.5
2	49.5
3	49.0
	$\bar{x}$ = 49.7
	Std. Dev. = 0.80

$$O_2 \text{ permeability} = 49.7 \text{ mV} \times \frac{100 \text{ cc/m}^2\text{/24 hrs.}}{1 \text{ mV}} = 4{,}970 \text{ cc/m}^2\text{/day}$$

Saran (2 mil)

sensitivity = 10 mV full scale

53 Ohm resistor: $\frac{10 \text{ cc/m}^2\text{/24 hrs.}}{\text{mV}}$

Runs made at 32°C and 0% RH.

Table C2
Saran mV Response

Run No.	mV Response
1	1.3
2	1.3
3	1.4

$\bar{x}$ = 1.3
Std. Dev. = 0.10

O_2 permeability = 1.3 mV x 10 $\frac{cc/m^2/24\ hrs.}{mV}$ = 13 $cc/m^2/day$

Saran coated nylon (0.75)

sensitivity = 10 mV full scale

53 Ohm resistor: $\frac{10\ cc/m^2/24\ hrs.}{mV}$

Runs made at 32°C and 0% RH.

Table C3
Saran coated nylon mV Response

Run No.	mV Response
1	1.8
2	1.7
3	1.7

$\bar{x}$ = 1.7
Std. Dev. = 0.10

O_2 permeability = 1.7 mV x $\frac{10\ cc/m^2/24\ hrs.}{mV}$

Carbon Dioxide (Permatran-C)

The methods used to analyze the film samples for carbon dioxide, were identical to those suggested by Modern Controls Co.. The saran and saran coated nylon samples were tested using the good barrier method, where the polyethylene sample was tested with the poor barrier

procedure. The CO_2 transmission rate can be calculated using the following equation.

$$CO_2 \text{ Transmission Rate} = \frac{\text{capture volume (cc)}}{\text{time to reach calibration point (minutes)}} \times \frac{1440 \text{ min.}}{\text{day}} \times \frac{10^4 \text{ cm}^2/\text{m}^2}{\text{surface area } (\text{cm}^2)}$$

Polyethylene (2 mil)

5 V full scale
chart speed - 4 inches/hour
surface area - 5 cm^2
capture volume - 0.02 cc
calibration response - 1.8 units

Table C4
Calibration Response Time (Polyethylene)

Trial #	Calibration Response Time (min.)
1	2.7
2	2.7
3	2.7

$$CO_2 \text{ Transmission Rate} = \frac{0.02 \text{ cc}}{2.7 \text{ min.}} \times \frac{1440 \text{ min.}}{\text{day}} \times \frac{10^4 \text{ cm}^2/\text{m}2}{5 \text{ cm}^2}$$

$$= 21{,}333 \text{ cc}/\text{m}^2/\text{day}$$

Saran coated nylon (0.75 mil)

500 mV full scale
chart speed - 4 inches/hour
surface area - 50 cm^2
capture volume - 0.002 cc
calibration response - 1.8 units

Table C5
Calibration Response Time (Saran coated nylon)

Trial #	Calibration Response Time (min.)
1	8.5
2	8.5
3	8.5

$$CO^2 \text{ Transmission Rate} = \frac{0.002 \text{ cc}}{8.5 \text{ min.}} \times \frac{1440 \text{ min.}}{\text{day}} \times \frac{10^4 \text{ cm}^2/\text{m}^2}{50 \text{ cm}^2}$$

$$= 67.8 \text{ cc}/\text{m}^2/\text{day}$$

Saran (2 mil)

500 mV full scale
chart speed - 4 inches/hour
surface area - 50 cm^2
capture volume - 0.002 cc
calibration response - 1.8 units

Table C6
Calibration Response Time (Saran)

Trial #	Calibration Response Time (min.)
1	10.5
2	10.5
3	10.5

$$CO^2 \text{ Transmission Rate} = \frac{0.002 \text{ cc}}{10.5 \text{ min.}} \times \frac{1440 \text{ min.}}{\text{day}} \times \frac{10^4 \ cm^2/m^2}{50 \ cm^2}$$

$$= 54.8 \ cc/m^2/day$$

Appendix D

Calculation of Solubility Constants for O_2 and CO_2

Appendix D

Calculation of solubility constants for O^2 and CO^2

A linear interpolation of the solubility constants for O_2 and CO_2 at 32°C was obtained by using the values at 30°C and 35°C.

O_2 solubility = 0.02608 - [(0.02608 - 0.02440) x 2/5]
coefficient = 0.02541 cc/ml at 32°C and 1 atm of gas pressure

CO^2 solubility = 0.665 - [(0.665 - 0.592) x 2/5]
coefficient = 0.650 cc/ml at 32°C and 1 atm of gas pressure

Note: Solubility constants obtained from the text (CRC, 1936) are given on a volume gas/volume water basis at their respective temperatures and at a pure gas pressure of 1 atm.

Appendix E

Buffer Formula

Appendix E

Buffer Formula

3.631 g	KH_2PO_4
7.125 g	$K_2HPO_4(3H_2O)$
1 liter	H_2O

0.05 Molar

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