THE KINETICS OF GLUCOSE LIMITED GROWTH BY A MARINE YEAST

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THE KINETICS OF GLUCOSE LIMITED GROWTH BY A MARINE YEAST

A

DISSERTATION

Presented to the Faculty of the
University of Alaska in Partial Fulfillment
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ABSTRACT

The kinetics of glucose limited growth by a marine yeast, shown to be a Rhodotorula species, have been studied in a continuous culture apparatus. The saturation constant, in synthetic media, has been calculated to be 0.25 mg/l, on the assumption that saturation kinetics are followed. The maximum growth rate was determined in both synthetic media and artificial sea water. On the basis of inhibition kinetics, the kinetic behavior of this yeast in the marine environment has been predicted.

The effect of temperature on the maximum growth rate has been determined and, on the assumption of a similar effect on the saturation constant, the saturation constant has been postulated to be in agreement with similar values determined for other microorganisms.

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INTRODUCTION

During the last several years much attention has been focused on the role of microorganisms in the chemical balance of the marine environment (Hamilton, Morgan, and Strickland, 1966; Jannasch, 1967; Vaccaro and Jannasch, 1966-1967; Wright and Hobbie, 1965-1966). While these investigations have demonstrated the interdependence of standing concentrations of dissolved nutrients and nutrient removal rates by microorganisms, there still exists no general numerical model for nutrient uptake in marine systems. The investigations of glucose incorporation by a marine yeast under conditions of nutrient limitation and low temperature was prompted by the need for a term describing nutrient flux under these conditions in such a model.

Some data describing nutrient flux under similar conditions is available for bacteria (Hamilton, Morgan, and Strickland, 1966; Jannasch, 1967; Vaccaro and Jannasch, 1966-1967) and natural populations (Vaccaro and Jannasch, 1966-1967; Wright and Hobbie, 1965-1966). This data is, however, mainly the result of short-term batch culture experiments. Continuous culture technique has been suggested to be more suitable for the determination of kinetic constants since it provides a constant environment over long

periods of time (Herbert, Ellsworth, and Telling, 1956) which results in a more physiologically uniform culture (Ieruselimsky, 1964). Jannasch (1967) has also suggested that the constancy of this environment renders this technique especially useful for investigation of marine organisms, since the marine environment may be expected to remain constant for long periods of time relative to the generation times of the microorganisms present. This similarity between the conditions in a continuous culture apparatus and the marine environment is not complete as mixed cultures in a continuous culture apparatus are subjected to a selection for microorganisms having a high maximum growth rate μ_{max} and a low saturation constant $K_{0.5}$ which is more rigorous than that in the marine environment (Hamilton, Morgan and Strickland, 1966).

It is noteworthy that the one investigation of marine systems which has employed the continuous culture technique (Jannasch, 1966) has yielded results which are not consistent with commonly accepted models of nutrient limited growth. This inconsistency has been interpreted to suggest a inhibitory effect linked to population density, however, the exact cause remains undetermined.

While a number of alternative expressions have been proposed for relating microbial growth rate to limiting nutrient concentration (Kono, 1968, Pena, 1965), the

Michaelis-Menten equation remains, if not the most correct, the most usable. Deviations from Michaelis-Menten behavior have been observed, however, this expression will reliably indicate the concentration region in which nutrient limitation of growth rate takes place. It should be noted that, while the expressions describing continuous culture were originally formulated from the Michaelis-Menten equation, any mathematical model which relates specific growth rate to limiting nutrient concentrations in an increasing non-periodic fashion may serve as a basis for continuous culture.

The general theory of continuous culture is well known and has been widely reviewed (Herbert, Ellsworth, and Telling, 1956; Malek, 1964; Malek and Fencl, 1966). The procedure of predicting limiting nutrient concentration S at a given growth rate μ from the population X, the input nutrient concentration S_o and the yield constant γ as suggested by Rao (1962) by extrapolating a plot of the equation $X=\gamma(S_o-S)$ to zero cell population has been questioned. Johnson (1967) measured oxygen concentrations in an oxygen limited culture of _C. utilis_ and found values somewhat lower than those predicted by Button and Garver (1966) using this method. The significance of this difference is difficult to evaluate due to differences in the pH and composition of the media employed in the two investigations. Investigation into amino acid limitation of

growth rate (Davies, Karush, and Rudd, 1965) has provided independently determined nutrient concentrations which agree quite well with the predicted values. This method of predicting nutrient values must therefore remain open to question, pending further investigation.

The independent determination of nutrient concentrations in the range over which nutrient limitation takes place is difficult for most substances. In the case of glucose limitation this concentration is on the order of 1 mg/l and is below the optimum range of any sufficiently specific chemical procedures which were available at the time this investigation was begun. Recently, Hicks and Carey (1968) have developed an enzymatic procedure suitable for this concentration range. In the current investigation C^{14} glucose was added to the media and glucose concentration in the spent media determined by counting after removal of cells by filtration. Since this procedure is not specific for glucose, it was necessary to check for the presence of excreted C^{14} metabolites by gas and paper chromatography.

A psychrophilic yeast, rather than a bacterium, was chosen for this investigation, since both types of organisms exhibit kineticly similar behavior, as shown in Table 1, and yeast have proven to be more emenable for long term continuous culture due to their lack of thigmotactic tendencies.

TABLE I

Organism	Limiting Nutrient	μ (hr^{-1})	S (mg/l)	Temp. (°C)	Reference
C. utilis	oxygen	0.08	0.23	35	Borkowski & Johnson, 1967
M. roseus	oxygen	0.08	0.23	25	Borkowski & Johnson, 1967
C. utilis	glucose	0.25	3.00	25	Button, 1968
E. coli	glucose	0.42	3.78	35	Rao, 1962
C. utilis	glycerol	0.25	4.5	35	Button & Garver, 1966
C. utilis	glycerol	0.075	1.44	18	Pena, 1965

MATERIALS AND METHODS

Apparatus   The continuous culture apparatus used was similar to that of Button and Garver (1966), and consisted of a 1 L Pyrex round bottom flask with a shortened neck, a Milton Roy model CH "miniPump," and a 12 L Pyrex feed carboy. The feed carboy was positioned slightly above the culture flask and aerated with filtered air to provide both the necessary oxygen and a slight positive pressure (~5 cm of Hg). It was found that this pressure assisted in maintaining a constant flow rate, provided sufficient back pressure on the pump was maintained.

At approximately 8 hour intervals, the pump was connected to a supply of concentrated KOH solution through a three-way stopcock positioned between the culture vessel and pump, and allowed to pump at maximum pumping rate for 10-15 minutes. The pump was then reconnected to the culture vessel after reducing the flow rate to the desired value. Immediately following this, the flow rate was measured by collecting the effluent for a fixed time and any minor adjustments made. The flow rate was also measured at irregular intervals between cleanings and the variation found to be less than 1%, provided the cleaning schedule was followed.

The culture vessel was surrounded by an insulated

bath containing an ethylene glycol-water refrigerant pumped from a refrigerated constant temperature bath-circulator (Forma-temp Refrigerated Bath-Circular, Constant Temperature; Forma Scientific Inc., Marietta, Ohio). Temperatures were reliably controlled to within 0.1 C. The culture vessel was agitated by a Teflon coated magnetic stirring bar. Stirring speed was not measured, however, dye solutions were observed to be completely dispersed within 0.5-1.0 seconds after injection.

Samples were withdrawn with a sterile hypodermic syringe inserted through a rubber stopper in the mouth of the culture vessel. Routine population determinations were made with a particle counter as well as viable counts.

Media   The media employed for continuous culture, referred to as synthetic media, was specifically designed for continuous culture of yeast at low populations. All nutrients with the exception of the limiting nutrient were present in appropriate amounts to avoid nutrient limitation or inhibition of the growth rate (Button and Garver, 1966). It contained (all concentrations per liter) $NaH_2PO_4 \cdot H_2O$ 2.00g, $(NH_4)_2SO_4$ 0.60 g, KCl 40.0 mg, $MgSO_4$ 24.0 mg, $CaCl_2 \cdot H_2O$ 5.0 mg $ZnS)_4 \cdot H_2O$ 0.7 mg, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ 0.5 mg, $CuSO_4 \cdot 5H_2O$ 25.0 μg, $MnSO_4 \cdot H_2O$ 1.5 μg, MoO_3 0.5 μg, biotin 1.0 μg, thiamine 1.0 μg, and B_{12} 1.0 μg.
For continuous culture work the media was prepared with

filtered distilled water, the pH adjusted to 4 and autoclaved at 130°C for 30 minutes. Glucose was then added from a filter sterilized stock solution. The C^{14} glucose was added from a separate sterile stock solution to enable both the glucose concentration and the specific activity to be varied as desired. A pH of 4.0 was chosen to lessen the possibility of bacterial contamination since the particular yeast under investigation was known to be relatively insensitive to pH. Innocula were prepared in shake flask culture using the same media.

Plate count media contained, in addition to the media listed above, 0.5 g glucose and 17.0 g agar, which were added previous to autoclaving. The pH of the plate count media was adjusted to 7 before autoclaving, to prevent hydrolysis of the agar, and readjusted to pH 4 with sterile HCl afterward. At irregular intervals plate count media was prepared containing yeast or malt extract in addition to the above to serve as a check on selectivity of the plating procedure.

Three different media were used in the determination of maxium growth rates. Synthetic media was prepared containing 0.5 g glucose. The other media prepared used as a basis Rila Marine Mix (Rila Products, Teaneck, New Jersey) prepared according to their instructions. The media referred to as Rila + #1 contained in addition to Rila salts

glucose 0.5 g, $(NH_4)_2SO_4$ 0.5 g, biotin 1.0 μg, thiamine 1.0 μg, and B_{12} 1.0 μg and had a pH of 7.2. Media Rila + #2 contained in addition to Rila + #1 $NaH_2PO_4 \cdot H_2O$ 2.00 g and had a pH of 4.0. Special media used in the identification of the yeast were prepared in accordance with standard works on yeast identification (Difco, 1953; Lodder and Kreger-Van Rij, 1952; Wickerham, 1951).

All chemicals used were reagent grade. Uniformly labelled C^{14} glucose was obtained from Schwarz BioResearch in water-ethanol solution. The sample was evaporated, under reduced pressure, to dryness under sterile conditions and redissolved in filtered distilled water.

Maximum Growth Rate The maximum growth rates were determined in batch culture in the media previously described. A 1 L Pyrex flask was placed in the refrigeration apparatus used for continuous culture and stirred with a magnetic stirrer. Oxygen was supplied by aerating the culture vessel with filtered air. Populations were determined by measuring absorbance at 625 mμ of a sterilly removed sample in a 5 cm cell. Innocula were supplied from shake flask cultures incubated under similar conditions.

Organism The yeast under investigation was a Rhodotorula species similar though not identical to *Rhodotorula mucilaginosa* (Lodder and Kreger-Van Rij, 1952). It was

isolated by S.D. Burton south of Hawaii from a water sample taken in an ethanol sterilized Nasen bottle from 3800 meters (T = 1.6°C). Mature cells varied from 3μ to 5μ in width and from 5μ to 7μ in length. Reproduction was observed to be exclusively vegetative with budding usually axial although exceptions were observed. Thick suspensions or colonies on solid media were pink in color. Colonies were round convex with smooth to mucoid surface and a smooth to curled edge.

The organism was found to be quite nonspecific in its ability to utilize carbon sources, showing no growth on only ethanol and glycerol of the large number of alcohols, organic acids, esters, monosaccharides and disaccharides tested. Nitrate, ammonia, and organic amines were all found to be suitable sole nitrogen sources. All four serial dilutions in vitamin free media, growth could still be observed although at a considerably lessened level.

Population Determination Since it was felt desirable to check for the presence of non viable cells, particle counts were made daily with a Coulter Counter,(Model B, Coulter Electronics, Inc., Hialeah, Florida) using a 100μ orifice in addition to viable counts. A 10 ml sample was diluted with an equal volume of filtered 2% NaCl and four ½ ml counts made. In all cases background was checked and corrected for. Settings on the instrument corresponded

to a minimum cell volume of 7.58 μ^3. Maximum setting was set on lock so that the maximum particle volume detected was limited only by the orifice size and the linearity of the electronics.

Chromatography   Analysis for volatile excreted metabolites was carried out on an F & M Model 500 gas chromatograph (F & M Scientific Corp., Avondale, Pennsylvania) employing a thermal conductivity detector. Columns using Carbowax 600 (J. T. Baker Chemical Company, Philadelphia, Pennsylvania) or Apiezon L (F & M Scientific Corp., Avondale, Pennsylvania) as a liquid phase were used at temperatures ranging from 90°C to 150°C at various flow rates. Samples were prepared for gas chromatography by filtering batch cultures grown on synthetic media.

Analysis for nonvolatile excreted metabolites was by descending paper chromatography in H_2O - n-butanol - pyridine (1:2:2). Samples were prepared for chromatography by filtering the contents of the culture vessel, adding $HgCl_2$ to the filtrate, and heating to 100°C for 5 minutes. The sample was then concentrated to approximately 2 ml under reduced pressure in a rotary evaporator. The concentrate was stored at -10°C for three weeks to allow the maximum amount of salt to crystallize out. Three 10 μL samples of concentrate, C^{14} glucose media, and a 1% chromatogram was developed and dried.

The dried chromatogram was cut into strips 4 cm wide. The strip containing the 1% glucose was treated with $AgNO_3$-ethanol. The strips containing either the C^{14} glucose or spent media samples, were cut into 4 cm squares and counted in a liquid scintillation counter.

C^{14} Counting   All counts were done on a Nuclear-Chicago Model 6860 Mark 1 liquid scintillation counter (Nuclear-Chicago Corp., DesPlaines, Illinois). This instrument has three channel capabilities and approximates 4π geometry. Windows were set for simultaneous C^{14} and H^3 counting with the third channel used for quenching. All samples were corrected for quenching by the channels ratio method after subtracting background.

Samples were prepared for scintillation counting by filtering a 1 ml sample of spent media through a membrane filter (type HAWP, 13 mm Millipore Corp., Bedford, Mass.) and washing with a 1 ml portion of nonradioactive media. A 1 ml portion of the filtered and washed sample was evaporated onto a 1.25 cm square of Whatman #1 filter paper. Total time elapsed between sampling and preparation of the dried sample was kept to a minimum (~5 min) to minimize degradation of the glucose after sampling.

The filter paper was dried and placed in a Nuclear-Chicago liquid scintillation counting vial. The filter paper was supported in the vial by a stainless steel common

pin pushed through the center of the paper prior to evaporating the sample. The membrane filter was also placed in a counting vial and 2 ml of ethyl acetate added to dissolve the filter and then evaporated. Fifteen ml of counting solution consisting of 4.9 g of 2,5-diphenyloxazole and 0.1 g of 2,2'-p phenylenebis-(5-phenyloxazole) in toluene was added and the vials placed in the counter. All samples were counted a minimum of four 10 minute counts. After correcting for background, quenching, and dilution, glucose concentrations were determined by comparison with previously prepared standards. Quenching samples were prepared by counting identical samples of media with varying amounts of CH_3Cl added. Separate quenching samples were prepared for dissolved glucose and cellular C^{14} samples.

RESULTS

Gas chromatographic analysis of spent media from batch cultures in various growth phases and at various glucose concentrations showed the presence of ethanol in all cases. No other volatile metabolites were detected, although the presence of CO_2 may be assumed. Paper chromatography showed the presence of radioactivity in the spent media only in the area having a similar R_f value to that of glucose standards in the parallel chromatograms. Since the chromatogram was counted in discrete segments, it is possible that other compounds were present, since any compound having an R_f value within 10% of the R_f value for glucose would have been detected as glucose. Tabulated R_f values (Lederer and Lederer, 1957) of the various compounds which might be expected as excretory products suggest that these would fall outside the segment containing glucose. The sensitivity of both the gas and paper chromatographic procedures would allow the detection of less than 1% of the total carbon source. Since both the ethanol and CO_2 would be removed from the sample in the drying step previous to counting, it was assumed that all detected C^{14} was in the form of glucose and all concentrations calculated on that basis.

Two continuous culture runs were made, each lasting approximately 25 generation times, both at a dilution rate (D = flow rate/culture vessel volume) of 0.0132 hr^{-1} at input nutrient concentrations of 0.5 g and 1 mg/l glucose respectively. Population and nutrient concentrations at steady state are shown in Figures 1 and 2. Average population, as determined by viable counts, and average culture/vessel nutrient concentrations are shown in Table II.

TABLE II

Run	Population (cells/liter)	Nutrient Concentration (mg/l)
1	2.17×10^7	0.24
2	6.59×10^7	0.25

These values may be expected to be within 0.15 mg/l of the actual values, the major possibility of error lying in the preparation of the sample for scintillation counting. Figure 3 shows the variation of cell population with input nutrient concentration.

The effect of temperature on the maximum growth rate is shown in Figure 4. The energy of activation calculated from the Arrhenius equation is 1.99×10^4 cal/mol. Several attempts were made to measure the maximum growth rate

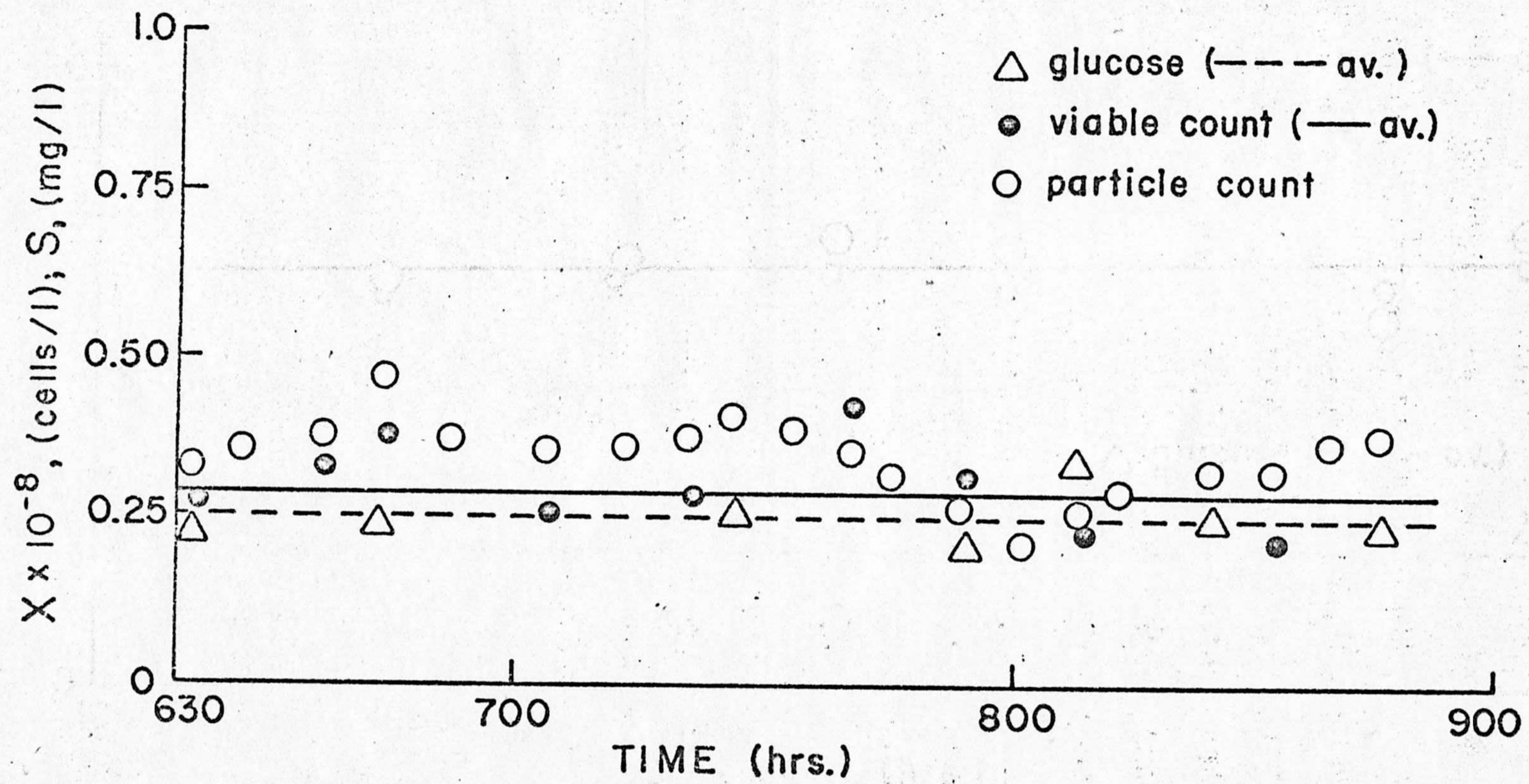

Fig. 1. Population and Nutrient Concentration, Run 1

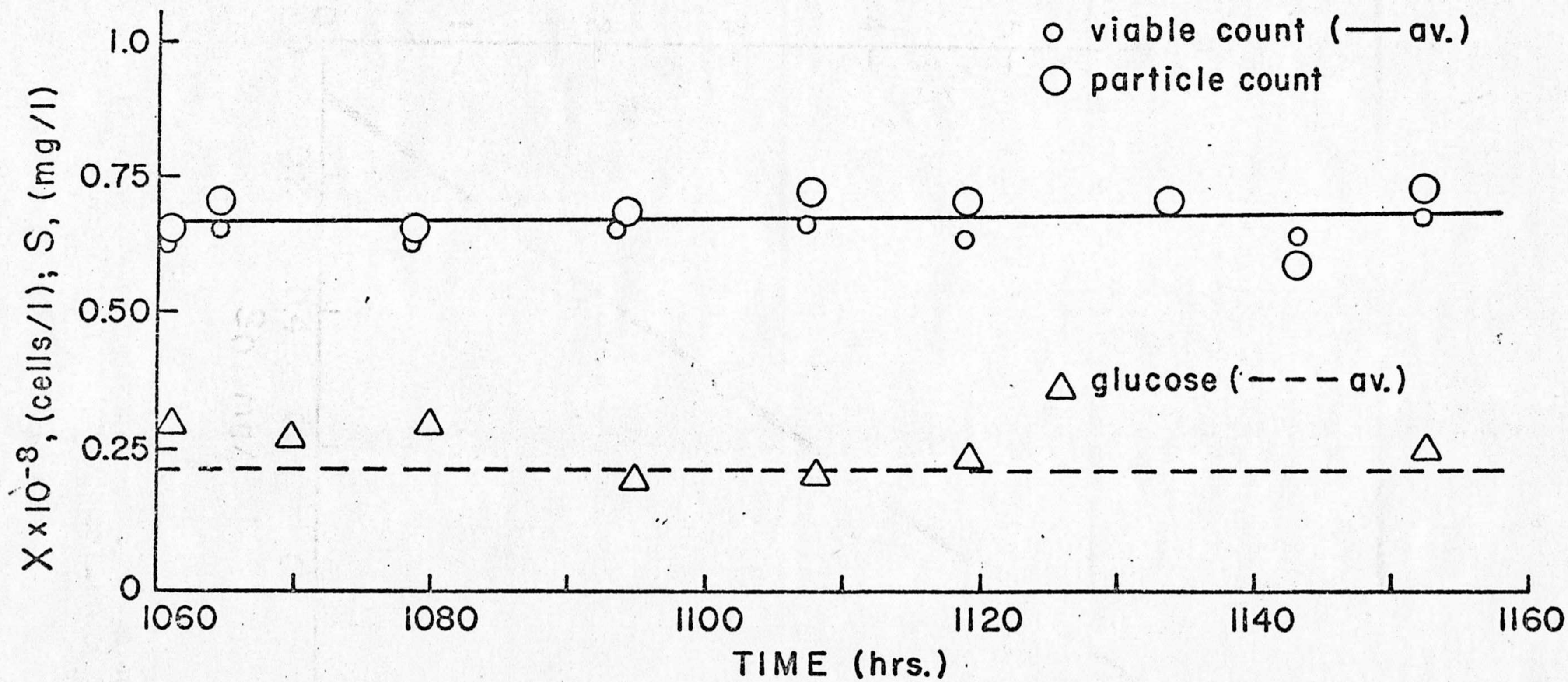

Fig. 2. Population and Nutrient Concentration, Run 2

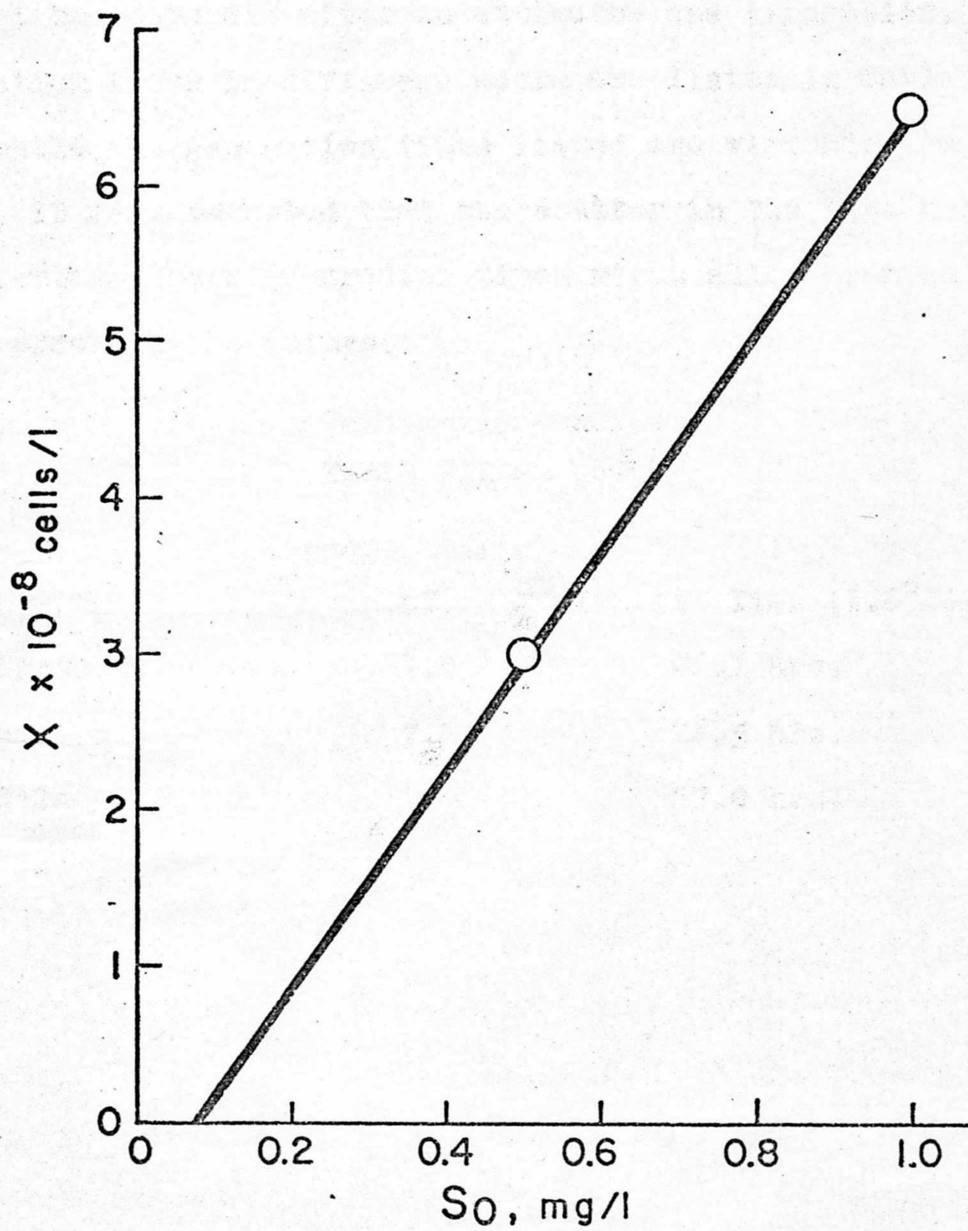

Fig. 3. Relationship of Population to Nutrient Concentration

at temperatures above 18°C, however, at 20°C no growth was observed and at 18.6^{o}C growth was erratic and the culture began to die after an estimated one generation. Generation times in different media are listed in Table III and, while the generation times listed are virtually identical, it must be noted that the scatter in the data used to calculate these generation times might allow as much as a 10% error in the values.

TABLE III

Media	pH	Generation Time (1.6^{o}C)
Synthetic media	4.0	26.1 hrs.
Rila + #1	7.2	26.5 hrs.
Rila + #2	4.0	27.0 hrs.

DISCUSSION

The temperature dependence of the organism under investigation would appear to be unusual. The high energy of activation suggests a mesophilic or even thermophilic character, while the temperature optimum around 18°C is in accordance with the definition of an obligate psychrophile (Morita, 1965).

At 1.6°C the observed generation time in synthetic media of 26.1 hr. gives a maximum growth rate of 0.0265 hr^{-1}. The dilution rate used is half this value. Flux calculations based on Fick's second law and employing the observed population and nutrient levels show that a nutrient concentration gradient in excess of 100 microns would be necessary to produce a diffusion limited system at the concentrations employed. This is in agreement with the calculations of Borkowski and Johnson (1967).

While itiis not possible to demonstrate saturation kinetics from the data at hand, it is reasonable to postulate, by analogy to other nutrient limited systems, that the system follows saturation kinetics and to calculate the saturation constant using the Michaelis-Menten equation. Since the culture was growing at half maximal velocity, the saturation constant is equal to the observed concentrations and therefore has an average value of 0.25 mg/l.

This value differs somewhat from the value predicted from a plot of population versus input nutrient concentration ($K_{0.5}$=0.09 mg/l), however this difference is probably not significant in light of the possible errors involved in the use of viable counts as a measure of cell mass.

The observed value of $K_{0.5}$ at 1.6°C is approximately one order of magnitude lower than the saturation constants observed for other organisms at higher temperatures as shown in Table IV.

TABLE IV

Organism	Limiting Nutrient	Temp. (°C)	$K_{0.5}$ (mg/L)	μ_{max} (hr^{-1})	Reference
C.utilis	glucose	25	3.00	.506	Button, 1968
C. utilis	glycerol	35	4.5	.506	Button and Garver, 1966
C.utilis	glycerol	18	1.44	.149	Pena, 1966
E. coli	glucose	35	3.78	.84	Rao, 1962

Cirillo (1962) has also estimated $K_{0.5}$ for glucose uptake in S. cerevisiae, however his estimate of 10^{-3}M was made from the concentration of glucose inhibiting sorbose uptake by 50% rather than by direct measurement. The range of these values may be explained by variations in technique and interspecific differences. Pena, however, noted that the slope of a plot of ln $K_{0.5}$ versus 1/T was identical

to that of an Arrhenius plot for C. utilis. While no theoretical justification is offered, it is noteworthy that values extrapolated by this method (Table V) from the value observed at 1.6°C are in general agreement with the literature values in Table IV.

TABLE V

Temp. (°C)	μ_{max} (hr^{-1})	$K_{0.5}$ (mg/L)
1.6	0.0265	.25
10	0.077	.72*
18	0.174	1.70*
25	0.379*	3.94*

*Predicted values

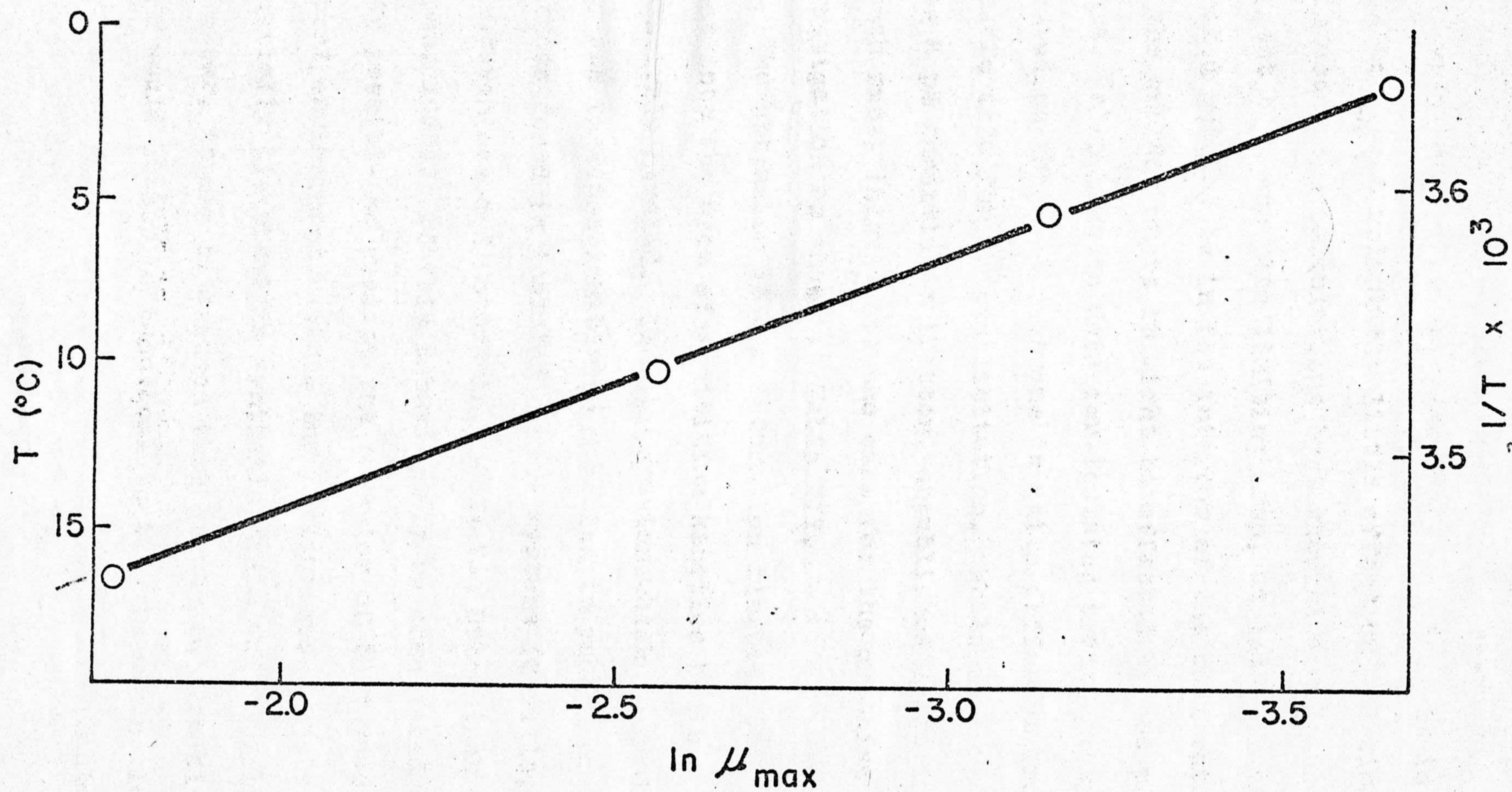

Fig. 4. Relationship of Maximum Growth Rate to Temperature

While the pH of the media used was low, it is reasonable to expect relatively little effect on the kinetics on this account. Calculations by Borkowski and Johnson (1967) suggest that the rate limiting step, at least in oxygen limited growth, is in the interior of the cell rather than at the surface where it might be effected by the pH of the media. Also kinetic data for isolated transport systems located on the cell membrane is quite different from the kinetic data for growth limitation. While some yeasts do show a pH sensitivity (Button, unpublished data) in the growth rate, this is not the case for the organism under investigation as shown in Table III.

The extension of the saturation kinetics observed in yeast into the area of inhibition kinetics has not been extensively studied. The information which is available suggests that the inhibition kinetics in yeasts are similar to those found in isolated enzyme systems (Cirillo, 1962; Van Steveninck and Rothstein, 1965; Van Steveninck and Dawson, 1968). If this behavior may be considered general, it is possible to predict the behavior of this yeast in its natural environment. Since the maximum growth rates are essentially identical in synthetic media and artificial sea water, it may be assumed that growth in natural sea water would either be identical to that in synthetic media or would be subject to competitive inhibition (Mahler and

Cordes, 1966). Competitive inhibition involves the competition, by a nonmetabolizable substance, at the site of the rate limiting step. Since this results in an increase in the effective $K_{0.5}$ and therefore a decrease in the specific growth rate produced by a given nutrient concentration, it is possible to predict the maximum specific growth rate which might be expected under marine conditions.

Jannasch, et al. (1968) have measured glucose concentrations in Atlantic waters and found from less than 2 µg/l µp to 195 ug/l. On the basis of these concentrations, a minimum generation time of 66 hours might be expected with an average generation time of 288 hours somewhat more likely. This assumes that the organisms utilizes glucose as a sole carbon source. These generation times are sufficiently long, particularly in light of the range of carbon sources the yeast is capable of assimilating, to indicate that the yeast probably utilizes a range of carbon sources under marine conditions.

APPENDICES

CONTINUOUS CULTURE THEORY

The kinetic basis of continuous culture can be most easily understood by consideration of the equations defining the population balance and the nutrient balance. The rate of change of population in the culture vessel is equal to the cell input plus the cells generated in the culture vessel minus the cells pumped out as shown in equation 1.

$$VdX/dt = FX_0 - FX + \mu XV \qquad (1)$$

Since the cell input in a single stage continuous culture apparatus is equal to zero the expression may be reduced as shown in equation 2.

$$VdX/dt = \mu XV - FX \qquad (2)$$

at steady state

$$dX/dt = 0 \qquad (3)$$

and dividing by V

$$0 = \mu X - FX/V \qquad (4)$$

therefore

$$\mu = F/V \qquad (5)$$

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Equation 5 demonstrates a method whereby the specific growth rate of a microorganism may be controlled by controlling the dilution rate, provided the dilution rate is greater than zero but less than the maximum growth rate.

The nutrient balance equation states that the rate of change of the limiting nutrient is equal to the input of nutrient minus the output of nutrient minus the output of nutrient in the form of cells as shown in equation 6.

$$V dS/dt = FS_o - FS - \mu XV/\gamma \qquad (6)$$

at steady state

$$dS/dt = 0 \qquad (7)$$

and dividing by V

$$0 = F/V\,(S_o - S) - \mu X/\gamma \qquad (8)$$

since $\mu = F/V$ from equation 5

$$X = \gamma(S_o - S) \qquad (9)$$

Rao (1962) has used equation 9 to predict the value of S by plotting the value of X at various input nutrient concentrations and extrapolating to zero cell population, however, this procedure is open to some question as previously discussed.

The derivation of these equations assumes four points;

(1) the specific growth rate is a function of the limiting nutrient concentration.

(2) all cells are viable.

(3) the environment in the culture vessel is homogenous.

(4) that steady state can be maintained.

Each of these points is subject to experimental verification by various methods, and each must be verified, as any appreciable deviation will effect the results.

Symbols used for Continuous Culture

Symbol	Definition	
X_o	Cell input into fermentor	(cells/volume)
X	Cell output from fermentor	(cells/volume)
F	Flow rate	(volume/time)
t	Time	(time)
μ	Specific growth rate	(1/time)
μ_{max}	Maximum growth rate	(1/time)
V	Volume of fermentor	(volume)
S_o	Nutrient input into fermentor	(mass/volume)
S	Nutrient input from fermentor	(mass/volume)
Y	Yield constant	(mass cells/ mass nutrient)

Additional Description of Rhodotorula sp.

Carbon assimilation tests were performed by the method outlined by Wickerham and used the media designed by him for this purpose.

Carbon Source	Growth	Gas production	Acid production
Acetate	3	-	-
Adonitol	2	-	-
Arabinose	2	-	-
Cellibiose	3	-	-
Citrate	3	neg.	neg.
Dulcitol	2	-	-
Erythritol	1	-	-
Ethanol	0	-	-
Ethyl acetoacetate	1	-	-
Galactose	3	pos.	pos.
Glucose	3	neg.	neg.
Glycerol	0	-	-
Inositol	2	-	-
Lactate	1	-	-
Lactose	1	neg.	neg.
Maltose	3	neg.	neg.
Mannitol	3	-	-
Mannose	3	-	-

Carbon Source	Growth	Gas production	Acid production
Melitzitose	3	-	-
Mellibiose	1	-	-
Methyl α D Glucoside	1	-	-
Raffinose	3	-	-
Rhamnose	2	-	-
Ribose	2	-	-
Salicilin	3	-	-
Sorbose	3	-	-
Succinate	3	neg.	neg.
Succhrose	3	pos.	pos.
Trehalose	3	-	-
Xylose	2	neg.	neg.

0 indicates no growth

1 indicates slow growth

2 indicates intermediate growth

3 indicates good growth

Several attempts were made to detect internal structure in the organism by various staining techniques, however, no capsule, fat globules, spores, or other internal bodies were observed.

Since the organism was isolated from a Nansen sample, the question of its origin must remain open. The

temperature characteristics, however, render it unlikely that the yeast was a contaminant, hence it is reasonable to assume it is marine in origin.

LIQUID SCINTILLATION QUENCHING CORRECTION

The phenomenon referred to as quenching in liquid scintillation counting is a collection of different processes whereby the energy detected by the counter from a given radioactive events is effectively decreased by inefficient energy transfer and interpreted by the scintillation counter as an event of lower energy. The actual mechanisms which produce this effect are generally understood from an experimental standpoint, though not from a theoretical one.

Since quenching can decrease the detected radiation by a variable and appreciable amount, it is necessary to either insure that quenching remains constant or correct the observed count for an experimentally determined degree of quenching. In systems for counting biological materials, where the substance being counted is of undefined chemical composition, it is preferable to correct for quenching, which necessitates a multi-channel counter.

The procedure used for quenching correction involves the determination of the number of radioactive events interpreted by the counter as having different energies. Since the quenching phenomenon involves the effective decrease in the energy of the radioactive event, the ratio of the events occurring in a high energy range to the events

occurring in a lower range is a measure of the quenching and may be related to the efficiency of counting for the high energy events. The actual procedure used for this correlation and the subsequent correction may be best understood by an example of the correction procedure. In the current investigation the channels or energy range limits, were set so that channel A corresponds to unquenched H^3 and C^{14} combined, channel B to H^3, and channel C to C^{14}. Since quenching of the higher energy C^{14} radiation could cause the C^{14} radiation to be interpreted as H^3 radiation, the degree of quenching and hence the efficiency of C^{14} counting may be expressed as a function of either the channels ratio C/A or C/B. If the relationships between the channels ratios and quenching have been determined by counting known samples with various amounts of a quenching compound added, the efficiency of a given count may be determined from this relationship. A sample calculation is given below.

Typical Tape Output

103	sample identification number
10	counting time in minutes
78110	total counts Channel A ($H^3 + C^{14}$)
57671	total counts Channel B (H^3)
13235	total counts CHannel C (C^{14})

Typical Tape Output

7811.00	counts per minute Channel A
5767.10	counts per minute Channel B
1323.50	counts per minute Channel C
0.7383	B/A
0.16944	C/A
0.22949	C/B

Since previous studies had shown the background to be independent of quenching, background corrections were made previous to quenching corrections. A Channel C background of 20.48 cpm was found for the run under discussion giving a total Channel C count of 1303.02 cpm. Comparison with previously prepared quenching standards gave a counting efficiency of 47.8% based on the C/A ratio and 47.9 based on the C/B ratio. Dividing by the calculated counting efficiency yielded a absolute counts of 2725.98 and 2720.29 respectively, for an average of 2723.12 dpm.

Similar calculations were made for all standards and samples and all standards and samples are average values for at least four 10 minute counts. While no statistical analysis was performed on the corrected data, accuracy may be expected to be within 5% or better.

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