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MICROBIAL CONVERSION OF GRAIN DUST TO USEFUL PRODUCTS

by

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CHAPTER I

INTRODUCTION AND SUMMARY

INTRODUCTION

This reserach is primarily concerned with the microbial utilization of grain dust for useful purposes. The yields which can be obtained when grain dust is utilized for the microbial production of protein and other useful substances are investigated. Samples of corn, wheat, and milo dusts are utilized in this investigation. This work is divided into three chapters, each of which is self contained. Tables are located at the end of each chapter.

Overview of Chapters

Chapter II is a review of the literature on the yield of microbial biomass on glucose and other carbohydrates as an extension of the work of Payne (1) and Payne and Weibe (2), to include the biomass energetic yield, η, of Minkevich and Eroshin (3). Also, consistency relationships and relationship among yields are presented, and growth yields which are corrected for maintenance and maintenance coefficient are reviewed.

Chapter III is an experimental investigation of the compositions and microbial utilization of corn, wheat and milo dusts. Starch in the dust samples is enzymatically hydrolyzed and used in shake flask experiments as filtered and unfiltered glucose for the cultivation of the yeast Candida utilis. The chemical composition of some of the dust samples and products is also measured. The estimated cost of the microbial protein product is compared to the current selling price of soybean meal, the main competitor.

Chapter IV is also an experimental study to evaluate the extent and efficiency of grain dust utilization by Candida utilis. Corn dust is selected for this study because it usually contains more starch than dusts from milo and wheat (see Chapter III). The enzymatically hydrolyzed starch in the corn dust sample is used in batch fermentation with and without filtration to remove the residual grain dust solids. Glucose concentration,

solid product/biomass concentrations, nitrogen concentration, oxygen consumption rate and carbon dioxide evolution rate are recorded and used to check the consistency of the data. The results of aerobic fermentation on both filtered and unfiltered glucose media are used to better understand how and to what extent the solid materials in the unfiltered media are utilized by microorganisms. The results of the experiments are used to estimate the growth yields and some growth related parameters. For the parameter estimation, statistical methods are developed.

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CHAPTER II

A REVIEW ON BIOMASS YIELDS AND MAINTENANCE REQUIREMENTS FOR GROWTH ON CARBOHYDRATES

INTRODUCTION:

The microbiological conversion of carbohydrates to cellular biomass and other useful products is a subject of increasing interest because of the increasing dependence on renewable resources. The basic technology for microbial cultivation of yeasts and molds on carbohydrates has existed for decades, and commercial practices are well established (1). There is a large volume of published literature on the microbial utilization of glucose and other carbohydrates (1-53).

The yield of microbial biomass on glucose and other carbohydrates is of significant interest because substrate costs are always a substantial portion of the operating costs in industrial plants. Several yield factors have been used in the literature and their validity has been critically reviewed by Payne (3) and Payne and Wiebe (4). One purpose of this review is to extend the work of Payne (3) and Payne and Wiebel (4) to include the biomass energetic yield, η, of Minkevich and Eroshin (5). A second purpose of this work is to present consistency relationships and relationships among yields. These relationships allow growth yields to be compared to maximum theoretical yields. The consistency relationships allow an evaluation of the accuracy of some of the data. Growth yields which are corrected for maintenance and maintenance coefficients are also reviewed. The consistency of independent estimates of growth yields, η_{max}, yields on ATP, Y_{ATP}^{max}, and adenosine triphosphate (ATP), production by substrate and oxidative phosphorylation are also reviewed.

THEORY

The yield factor Y_s is widely used and represents the mass of biomass produced per g of substrate consumed. The yields reviewed by Payne (3) and Payne and Wiebe (4) included: Y_{ATP} which is the yield of cells per mole of adenosine triphosphate (ATP) formed during growth; $Y_{ave.}$ which is the yield of cells per equivalent of available electrons in the substrate; Y_{kcal} which is the yield of cells per kilocalorie of total energy taken for biomass production, product formation, and heat liberation during growth.

Biomass Energetic Yield:

Recently, the biomass energetic yield, η, has been introduced and widely used in the literature (5-12). The biomass energetic yield, η, is the fraction of available electrons in the organic substrate which are incorporated into biomass. It is also approximately equal to the fraction of substrate energy which is incorporated into biomass (5,6,7). This dual significance of the biomass energetic yield occurs because the heat of reaction per equivalent of available electrons transferred to oxygen is approximately constant for a wide variety of organic compounds (5-8).

Two other regularities, the weight fraction of carbon in biomass, σ_b, and the equivalents of available electrons per g atom carbon, γ_b, are also required to relate the biomass energetic yield, η, to other yields. By analyzing 21 samples of yeast from production plants, Minkevich et al. (6) obtained an average weight fraction of carbon $\sigma_b = 0.446$. From elemental

analysis a reductance degree $\gamma_b = 4.209$ was estimated. Minkevich et al. (6) also obtained $\sigma_b = 0.477$ and $\gamma_b = 4.423$ for 12 reported values for bacteria in the literature. They obtained the pooled averages σ_b and γ_b, respectively as 0.462 and 4.291.

Minkevich and Eroshin (5) also found the heat of reaction per equivalent of available electrons transferred to oxygen, Q_0, to be 26.95 kcal/g equiv. of available electrons for conversion of biomass to CO_2, H_2O and NH_3. The coefficients of variation are 4% for Q_0 and γ_b, and 5% for σ_b (6).

Erickson (40) has examined some new data reported recently and compared the results with the average values of Minkevich and Eroshin. Some of the data provide further support for the average values reported by Minkevich and Eroshin; however, in the work of Ho and Payne (53) it is clear that in the halophiles and marine bacteria the weight fractions of carbon are much lower than 0.462. When grown on ammonia as the only source of nitrogen, microorganisms utilize different organic substrates according to the following stoichiometric equation

$$CH_mO_\ell + aNH_3 + bO_2 \rightarrow y_c\ CH_pO_nN_q + zCH_rO_sN_t + cH_2O + dCO_2 \quad (1)$$

In the above equation, CH_mO_ℓ represents the elemental composition of the organic substrate, $CH_rO_sN_t$ represents the average elemental composition of the extracellular products, and $CH_pO_nN_q$ the elemental composition of the biomass.

On the basis of this balanced equation we obtain the equation

$$\eta + \varepsilon + \xi_p = 1 \quad (2)$$

when available electron balance is made. Here,

$$\eta = \frac{y_c \gamma_b}{\gamma_s} = Y_s \frac{\sigma_b \gamma_b}{\sigma_s \gamma_s} \tag{3}$$

$$\varepsilon = \frac{4b}{\gamma_s} \tag{4}$$

$$\xi_p = z \frac{\gamma_p}{\gamma_s} \tag{5}$$

and η is the fraction of available electrons or energy in the substrate which is incorporated into biomass, ε the fraction of available electrons in the substrate which is transferred to oxygen and ξ_p the fraction of available electrons in substrate utilized in product formation.

In equation (1), y_c is the biomass carbon yield in units of mass of carbon in biomass per mass of carbon in the consumed organic substrate. A more commonly used yield is the mass yield Y_s, g biomass/g substrate consumed. From theoretical calculations Stouthamer (38) found that Y_{ATP}^{max} = 28.8 for the growth of microorganisms with glucose and inorganic salts. When 38 moles of ATP are generated from 1 mole of glucose, the maximum theoretical efficiency of biomass production under aerobic conditions is (12)

$$\eta_{max} = \frac{\frac{\sigma_b \gamma_b}{12} Y_{ATP}^{max}}{\frac{\sigma_b \gamma_b}{12} Y_{ATP}^{max} + \frac{2}{\alpha+\beta}} \tag{6}$$

This equation relates biomass energetic yield to Y_{ATP}^{max}.

Other yield factors of interest are $Y_{av.e}$ and Y_{kcal}. These yields are related to biomass energetic yield by the equations below.

$$\eta = \frac{\sigma_b \gamma_b}{12} Y_{av.e} \tag{7}$$

and

$$\eta = \frac{Y_{kcal} E_c \sigma_b \gamma_b}{\sigma_s \gamma_s} \tag{8}$$

when

$$E_c = \frac{\text{kcal substrate consumed}}{\text{g substrate consumed}}$$

However, if

$$E'_c = \frac{\text{kcal substrate consumed}}{\text{a.e substrate consumed}}$$

is available, then

$$\eta = \frac{E'_c \sigma_b \gamma_b}{12} Y_{kcal} \tag{9}$$

Maintence Requirements

Growing microorganisms requires a certain amount of energy for maintenance. This is required for the preservation of the right ionic composiiton, the turnover of cellular constituents and for several other processes needed to keep the organism alive. Pirt (18) has identified an equation which relates growth yields and the specific growth rate. The model can be written in the forms given below

$$Q_s = m_s + \frac{1}{Y_s^{max}} \mu \tag{10}$$

$$Q_{O_2} = m_O + \frac{1}{Y_O^{max}} \mu \tag{11}$$

$$Q_{CO_2} = m_D + \frac{1}{Y_D^{max}} \mu \tag{12}$$

and

$$\frac{\mu}{\eta} = m_e + \frac{1}{\eta_{max}} \mu \tag{13}$$

when there is no product formation and

$$\frac{\mu}{\eta}\left(1 - \frac{\xi p}{\xi_p^{max}}\right) = \frac{\mu}{\eta_{max}} + m_e \tag{14}$$

when there is product formation.

True growth yields can be obtained based on the organic substrate consumption, Y_s^{max}, the oxygen utilization, Y_0^{max}, the carbon dioxide production, Y_D^{max}, and the available electrons, η_{max}. The true growth yields can be related to the true biomass energetic field coefficient, η_{max}, as follows (11):

$$\frac{1}{Y_0^{max}} = \frac{\sigma_b \gamma_b}{48} \left(\frac{1}{\eta_{max}} - 1\right) \tag{15}$$

$$\frac{1}{Y_D^{max}} = \frac{\sigma_b}{12} \left(\frac{\gamma_b}{\gamma_s \eta_{max}} - 1\right) \tag{16}$$

$$\eta_{max} = \frac{\sigma_b \gamma_b}{\sigma_b \gamma_b} Y_s^{max} \tag{17}$$

Therefore, from substrate consumption, oxygen consumption and carbon dioxide measurements different estimates of true biomass energetic yields can be obtained (11); they can be compared to examine the consistency of the data.

The values of m_e, m_s, m_0 and m_D, which are, respectively, the energetic maintence coefficient, substrate maintence coefficient, oxygen maintenance coefficient, and carbon dioxide maintenance coefficient, are related as (11)

$$m_e = \frac{48}{\sigma_b \gamma_b} m_0 = \frac{12\gamma_s}{\sigma_b \gamma_b} m_D = \frac{\sigma_s \gamma_s}{\sigma_b \gamma_b} m_s \tag{18}$$

Data Consistency

It is important to properly consider the consistency of experimental measurements in estimating yield and maintenance parameters. Equation (2) may be used to check the consistency of experimental measurements. A carbon balance

$$y_c + d + z = 1 \tag{19}$$

may also be used. The consistency of parameter estimates of Parameters η_{max} and m_e may also be checked using Equations (15) - (18). Equation (6) may be used to examine the consistenty of independent estimates of η_{max}, Y_{ATP}^{max}, α, and β. When aerobic and anaerobic experiments are conducted with the same media and organism, values of Y_{ATP}^{max} and m_{ATP} may be estimated using the results obtained under anaerobic conditions and the relationship

$$\frac{\mu}{Y_{ATP}} = m_{ATP} + \frac{\mu}{Y_{ATP}^{max}} \tag{20}$$

Equation (11) may be used with the aerobic results to obtain estimates of m_0 and Y_0^{max}. The relationship

$$\frac{Y_0^{max}}{Y_{ATP}^{max}} = \frac{m_{ATP}}{m_0} = \alpha + \beta \tag{21}$$

is another consistency condition which may be employed.

ANALYSIS AND DISCUSSION

Yields:

Tables I,VI, VIII, and X contain the yield values for aerobically cultivated microoganisms with NH_4^+ as the source of nitrogen. Values of Y_s (g cell/g substrate), Y_{kcal} (g cell/kcal), and Y_{ave} (g cell/equivalent of available electrons) are obtained from the literature cited and are converted to biomass energetic yield using either equation (3), (7), (8), or (9) and the values of $\sigma_b = 0.462$ and $\gamma_b = 4.291$ reported by Minkevich and Eroshin (5) and Minkevich et al. (6). The consistency of the data used to calculate η in Tables VI and VIII were checked and they were found to be quite good. However, the consistency of the data in Tables I and X was not checked due to the lack of enough data. The average biomass energetic yield obtained from the results is 0.587. This implies that in aerobic fermentation of most microorganisms on carbohydrates with ammonia as the source of nitrogen, approximately 59% of the total energy in the substrate is incorporated into biomass. This value corresponds to $Y_{av.e} = 3.60$. Payne (3) in his review obtained a $Y_{av.e}$ value of 3.14 which, in turn, gives a value of the biomass energetic yield, η of 0.52 from equation (7).

Table VII contains the values of biomass energetic yields for Aerobacter aerogenes aerobically grown on glucose with KNO_3 as the nitrogen source (45). The average biomass energetic yield is 0.453. Compared to the average value for growth with ammonia as the source of nitrogen this value is low. This is due to the large amount of ATP required in utilizing nitrates in cell material synthesis. Thus in growth with KNO_3 as the source of nitrogen, some of the available electrons in the substrate are transferred to nitrogen, thereby reducing the total amount of available electrons which can be used in biomass formation.

Information on yield based on total amount of energy removed from the medium during growth is of great interest in microbial cultivation. This interest leads to the concept of Y_{kcal} (3), which is related to the biomass energetic yield by equations (8) and (9). The quantity of energy which is incorporated into biomass through biosynthetic process is denoted by E_a, and Mayberry et al. (36) have found the value to be 5.3 kcal/g dry wt. of cell. Using the relationship

$$Q_o = E_a / \frac{(\sigma_b \gamma_b)}{12} - 3.325 \qquad (22)$$

a value of Q_o = 28.76 kcal/eq. av. electron is obtained from the above E_a = 5.3 and σ_b = 0.462 and γ_b = 4.291. This value of Q_o is comparable to 26.95 kcal/eq av.e reported by Minkevich and Eroshin (5) and Minkevich et al. (6). However, in equation (22) correction has been made for the biomass nitrogen which is combusted to N_2 instead of NH_3.

Several workers obtained values of Y_{ATP} in close agreement with the 10.5 g cell/mole ATP postulated by Bauchop and Elsden (30). This value was accepted to be a universal constant until later workers (34, 35, 37, 38) obtained values of Y_{ATP} as high as 33 which has been reported to be theoretically possible (34). Some investigators have realized figures intermediate between 10.5 and the upper limit (37, 38). For microorganisms that grow anaerobically on substrates with known pathways, the relationship among substrate utilization, ATP generation and cell material formation is also known. Yields of organisms per mole of ATP after correction for maintenance, Y_{ATP}^{max}, are obtained using equation (20) (Pirt's Model). From theoretical calculations, Stouthamer (38) found Y_{ATP}^{max} = 28.8 for the growth of microorganisms with glucose and inorganic salts with ammonia as the source of nitrogen. When

38 moles of ATP can be generated per mole of glucose, P/O ratio or α, which is the moles of ATP formed by oxidative phosphorylation from ADP and inorganic phosphate per g atom oxygen, has a value of 3.0 and β, which is the moles of ATP formed by substrate level phosphorylation per g atom of oxygen, has a value of 0.167 (12). Under this situation application of equation (6) gives a value of $\eta_{max} = 0.88$. This implies that theoretically up to 88% of the energy or available electrons in glucose are incorporated into biomass when glucose is used as substrate. Experimental results indicate that the value of Y_{ATP} frequently lies between 8 and 13 even though much higher values can occasionally be found. For this range, values of η between 0.68 and 0.77 are to be expected, if the efficiency of oxidative phosphorylation and ATP utilization in the microorganism is the same as that which occurs in the mitochondria where $\alpha = 3.0$. The dependency of biomass energetic yield on Y_{ATP} and $\alpha + \beta$ is illustrated in Table IV. Using the average value of biomass energetic yield $\eta = 0.587$ and the average Y_{ATP} value of 10.5, the P/O ratio for the microorganisms is estimated to be 1.66. Jones (50) stated that due to low energy transduction efficiencies during microbial metabolism, P/O ratios are rarely greater than 1.0-1.5 for the oxidation of NADH. Also it has been shown that in heterotrophic cells phosphorylation site III is absent (51). For yeasts, a biomass energetic yield of 0.62 was obtained for growth on glucose and inorganic salts (1). This yield value corresponds to the P/O ratio of 1.88. The reviews of Jones (50) and Stouthamer (51) support the idea that $(\alpha+\beta)$ is around 2.0.

The results of the oxidative phosphorylation efficiency analysis using the results of Hadjpetrous (48) are shown in Table X. An example is given below the table to illustrate the procedure for carrying out this type of

calculation. On the average the efficiency of oxidative phosphorylation with respect to the maximum efficiency in the mitochondria is 66.1% for the growth of Aerobacter aerogenes on common carbohydrates. Using the value of this efficiency we can estimate that 25.8 moles of ATP are produced per mole of monomer. This value in conjunction with Y_{ATP} = 10.5 gives $\eta = 0.64$ from equation (6), which is in good agreement with values realized in the laboratory. It is, therefore, concluded that microorganisms frequently obtain less than 38 moles of utilizable ATP from 1 mole of glucose. This inefficiency in ATP generation and utilization leads to $\eta < 0.88$ in real situations.

In anaerobic fermentation of facultative aerobes ATP production is only by substrate level phosphorylation and thus only a few ATP can be produced per mole of any substrate. As such the growth yields during anaerobic processes are generally smaller than yields obtained in fully aerobic cultures. The results in Table II support the above statement. For yield values presented, an average energetic yield value of 0.184 is obtained compared with 0.587 and 0.580 for aerobic growth of pure cultures and mixed cultures, respectively. Thus, during anaerobic fermentations where electrons or hydrogen acceptors are absent, about 18.4% of the total energy in carbohydrates is incorporated into biomass; the rest is evolved as heat or utilized in the formation of products such as ethyl alcohol, acetate, lactic acid, propionic acid and polysaccharides.

Maintenance Requirements

Several models for estimating maintenance coefficients, true growth yields and true product yields have been developed by Erickson et al. (10, 11, 45, 44). To estimate true biomass energetic yield when there are no products formed can be easily done using equation (13). However, with product formation η_{max} is difficult to estimate and often ξ_p^{max} (true product yield) has to be used as parameter. Furthermore, the values of the yield parameters, η_{max} and ξ_p^{max}, may not be constant because of alternate biochemical pathways with different efficiencies (11,44). It has already been discussed that $\eta_{max} = 0.88$ is the theoretical maximum value of true biomass energetic yield when microorganisms are aerobically grown on glucose with ammonia as the source of nitrogen. A theoretical maximum value $\eta_{max} = 0.63$ is obtainable when KNO_3 is the source of nitrogen (45). To further support the idea that η_{max} and m_e depend on experimental conditions, Erickson (44) obtained values of $\eta_{max} = 0.77$ and $m_e = 0.018\ hr^{-1}$ for $\xi_p^{max} = 1.0$ under oxygen limited conditions but $\eta_{max} = 0.66$ and $m_e = 0.0070\ hr^{-1}$ were obtained under glucose limited conditions. For the aerobic growth of Aerobacter aerogenes on glucose with KNO_3 as the source of nitrogen (52), $\eta_{max} = 0.52$ and $m_e = 0.080\ hr^{-1}$, and $\eta_{max} = 0.43$ and $m_e = 0.133\ hr^{-1}$ were obtained by Erickson (45) under glucose and sulfate limited conditions, respectively.

Using ξ_p^{max} as parameter (10), η_{max} was found to about 0.7 for Aspergillus nidulans grown on glucose in chemostat experiments.

The value of η_{max} is dependent on whether products are formed or not. In an experiment where glucose was fermented to ethanol at different

dilution rates (41, 11), $\eta_{max} = 0.59$ and $m_e = 0.011\ hr^{-1}$ were obtained for the portion of the data in which ethanol was not formed. For the portion when ethanol was formed, values of η_{max} of 0.59 to 0.83 were obtained. Thus, η_{max} is larger when ethanol is produced and this is because of the higher energetic yield associated with high energy ATP bond formation by substrate level phosphorylation (11).

Table V contains the maintenance coefficients m_o, m_s and m_e, the true growth yields Y_o^{max} and Y_s^{max} and the true biomass energetic yield η_{max} for several microorganisms grown with glucose. The value of m_e was estimated from the m_o and m_s values using equation (19) and η_{max} was estimated from Y_o^{max} and Y_s^{max} using equation (15) and (17), respectively. The consistency of the estimates will be discussed later. However, in general low maintenance energy was obtained for glucose limited chemostat cultures compared to the sulphate, ammonium or oxygen limited cases. However, the result is not consistent enough to warrant any general conclusion. To maximize yield in glucose utilization by microorganisms, the culture must be properly aerated to ensure enough oxygen is available for complete oxidative phosphorylation to take place. Also the necessary inorganic elements for growth must be supplied in excess. However, the ionic strength of the medium should not be too high since it can lead to plasmolysis of the cells or high maintenance requirements. The average of η_{max} and m_e obtained for growth of several microorganisms on glucose are found to be 0.65 and 0.03 hr^{-1} respectively. Some of the values are presented on Table V and they are obtained from data which satisfy the consistency tests. Other values were obtained from (44, 43, 41, 11).

Consistency of Data

The importance of good data in the literature cannot be overemphasized, as the lack of it can lead to erroneous conclusions about microbial physiology. The concept of available electrons offers an easy but reliable method for checking the consistency of experimental data. Erickson et al., (9, 10, 11, 12, 40, 44, 45, 47) have applied this method to check the consistency of data in carbohydrate and hydrocarbon fermentations. Equations (2) and (19) can be used to check the consistency of experimental measurements. It is obvious that when $\eta + \varepsilon + \xi_p < 1$ and/or $y_c + d + z < 1$ then either an experimental error is involved or unmeasured products are produced. If, however, $\eta + \varepsilon + \xi_p > 1$ and/or $y_c + d + z > 1$, then it is definite that an experimental error is involved in the data collecting process.

For fermentation processes in which product formation is assumed to be unimportant, we have ($\xi_p = 0$, $z = 0$) and thus

$$\eta + \varepsilon = 1$$

and

$$y_c + d = 1$$

For the analysis, allowance of deviation from ideality is provided statistically based on the coefficient of variation of the regularities; the 95% confidence intervals are $0.94 \leq y_c + d + z \leq 1.06$ and $0.93 \leq \eta + \varepsilon + \xi_p \leq 1.07$. Several of the data already analyzed showed that considerable inconsistency exists in the data in the literature (9, 10, 12, 47). Tables VI, VII, and VIII contain examples of consistency examinations on some of the data in the literature using the principle discussed above. Another way to check the consistency of experimental data is to obtain different estimates of η_{max} and m_e from different sets

of data. For consistent data, the estimates should be the same. This method is illustrated in Table V where η_{max} was estimated from Y_s^{max} and Y_0^{max} using equations (3) and (5), respectively. The results in this table show that most of the data are not consistent since values of η_{max} estimated from Y_s^{max} are significantly different from values estimated from Y_0^{max} with the exception of a few cases. Values of m_e were estimated from m_s and m_o values using equation (19). For the growth of Aerobacter aerogenes under sulfate limited conditions, $\eta_{max} = 0.66$ and $m_e = 0.119\ hr^{-1}$, and $\eta_{max} = 0.29$ and $m_e = 0.174\ hr^{-1}$ were obtained from oxygen data and substrate data, respectively. This result is inconsistent since there is a significant difference between the different estimates. However, for the growth of E. coli under glucose limited conditions, $\eta_{max} = 0.63$ and $m_e = 0.073\ hr^{-1}$, and $\eta_{max} = 0.65$ and $m_e = 0.073\ hr^{-1}$ were obtained from oxygen and glucose data, respectively. This is an example of a consistent result.

Another way of checking the consistency of experimental data is by the use of the relationship in equation (21) (12). When anaerobic and aerobic experiments are conducted with the same media and organism, values of Y_{ATP}^{max} and m_{ATP} are usually estimated using the results under anaerobic conditions, equation (20), while Y_0^{max} and m_o can be estimated using the results of aerobic conditions, equation (11). The estimates must satisfy equation (21) if the data are consistent. Table IX tests consistency of this type. It can be seen that the data for growth on glucose are inconsistent since

$$\frac{m_{ATP}}{m_o} = 21$$

while

$$\frac{Y_0^{max}}{Y_{ATP}^{max}} = 1.21$$

In conclusion, the sources of error involved in data recording during fermentation are numerous. More disturbing still is the nature of the data which fails to lend itself to accuracy judgement just by the conventional statistical approach. There is therefore a need for checking the consistency of data collected. The method of material and energy balances illustrated in this thesis is a powerful, quick and straight forward way to do this. The employment of this method will reduce the accumulation of inaccurate data in the literature.

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NOMENCLATURE

a = Moles of ammonia per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon.

b = Moles of oxygen per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon.

c = Moles of water per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon.

d = Moles of carbon dioxide per quantity of organic substrate containing one g atom carbon g mole/g atom carbon.

ℓ = Atomic ratio of oxygen to carbon in organic substrate, dimensionless.

m = Atomic ratio of hydrogen to carbon in organic substrate, dimensionless.

m_{ATP} = Rate of ATP utilization for maintenance, g moles ATP/g biomass (hr).

m_D = Rate of carbon dioxide evolution for maintenance, g moles/g dry biomass (hr).

m_e = Rate of organic substrate available electron consumption for maintenance, g equiv./g equiv. of available electrons in biomass (hr) or kcal/kcal of biomass (hr).

m_o = Rate of oxygen uptake for maintenance, g moles/g dry biomass (hr).

m_s = Rate of organic substrate consumption for maintenance g/g of dry biomass (hr).

n = Atomic ratio of oxygen to carbon in biomass, dimensionless.

p = Atomic ratio of hydrogen to carbon in biomass, dimensionless

Q_{CO_2} = Rate of evolution of carbon dioxide, g moles/g dry wt (hr).

Q_o = Heat evolution in fermentation per equivalent of oxygen uptake, kcal/g equiv.

Q_s = Rate of organic substrate consumption, g/g dry wt (hr).

q = Atomic Ratio of nitrogen to carbon in biomass, dimensionless.

r = Atom ratio of hydrogen to carbon in products, dimensionless.

s = Atomic ratio of oxygen to carbon in product, dimensionless.

t = Atomic ratio of nitrogen to carbon in products, dimensionless.

$Y_{av.e}$ = Biomass yield based on available electrons, g dry biomass/g equiv.

Y_{ATP} = Biomass yield based on ATP, g dry biomass/g mole ATP.

Y_D^{max} = True biomass yield based on CO_2 evolution, g dry biomass/g mole.

Y_{kcal} = Biomass yield based on energy in substrate, g dry biomass/kcal.

Y_O^{max} = True biomass yield based on oxygen, g dry biomass/g mole.

Y_s = Biomass yield on organic substrate, g dry wt/g substrate.

Y_s^{max} = True biomass yield based on organic substrate, g dry wt/g substrate.

y_c = Biomass carbon yield (fraction of organic substrate carbon in biomass), dimensionless.

z = Fraction of organic substrate carbon in products, dimensionless.

γ_b = Reductance degree of biomass, equivalents of available electrons per g atom carbon.

γ_p = Reductance degree of products, equivalents of available electrons per g atom carbon.

γ_s = Reductance degree of organic substrate, equivalents of available electrons per g atom carbon.

ε = Fraction of energy in organic substrate which is evolved as heat, dimensionless.

η = Fraction of energy in organic substrate which is converted to biomass or biomass energetic yield, dimensionless.

η_{max} = True biomass energetic yield, dimensionless.

μ = Specific growth rate, hr^{-1}.

ξ_p = Fraction of energy in organic substrate which is converted to products, dimensionless.

σ_b = Weight fraction carbon in biomass, dimensionless.

σ_s = Weight fraction carbon in organic substrate, dimensionless.

Table I. Growth Yields for Aerobic Cultures.

Organisms	Reference	Medium	Substrate	Y_s g cell/ g sub.	Y_{kcal} g/kcal	Y_{ave}	η
Ripomyces starkeyi	(15)	Minimal	Starch	0.532	---	---	0.593
Candida utilis	(1)	"	Glucose	0.50	---	---	0.62
A. aerogenes	(16)	Minimal	Maltose	0.436	0.141	3.11	0.54
			Sucrose	0.505	0.144	3.60	0.626
			Mannitol	0.524	---	3.67	0.651
			Galactose	0.408	---	3.06	0.506
			Glucose	0.404	0.143	3.03	0.510
			Sorbitol	0.535	0.135	3.75	0.664
	(17)		Glucose	0.404	0.143	3.03	0.501
Arthrobacter globiformis	(18)	"	Glucose	0.520	---	3.92	0.64
Bacillus subtilis	(19)	Supple-mental	Glucose	0.451	---	3.39	0.560
Candida utilis	(20)	Minimal	Glucose	0.511	0.128	3.89	0.633
Candida utilis	(21)	"	"	0.504	---	3.78	0.625
Candida utilis	(22)	"	"	0.515	---	3.86	0.639
E. coli	(23)	"	"	0.377	---	2.83	0.467
E. coli	(24)	"	"	0.522	---	3.92	0.648
E. coli	(25)	"	"	0.50	---	3.72	0.620
Neurosporal crassa	(26)	"	Sucrose	0.336	---	2.40	0.420
Pseudomonas aeroginosa	(13)	Minimal 30°C	Glucose	0.434	0.116	3.25	0.540
		37°C	Glucose	0.423	0.114	3.18	0.530

Table 1. Growth Yields for Aerobic Cultures . . .

Organisms	Reference	Medium	Substrate	Y_s g cell/ g sub.	Y_{kcal} g/kcal	Y_{ave}	η
Pseudomonas fluorescens	(20)	Minimal	Glucose	0.386	0.111	---	0.48
	(27)	"	"	0.662	---	4.96	0.820
Saccharomyes cerevisiae	(41)	Supple-mental	Glucose	0.396	---	2.97	0.49
	(26)	"	"	0.435	---	3.27	0.539
	(1)	Minimal	Glucose	0.556	---	---	0.689
Asperigillus awamori	(1)	"	Glucose	0.528	---	---	0.654
Aspergillus nidulans	(1)	"	"	0.611	---	---	0.758
Penicillium chrysogenum	(1)	"	Glucose	0.483	---	---	0.599
Average Values				0.490	0.131	3.611	0.587

Table II. Growth Yields for Anaerobic Cultures.

Organism	Reference	Medium	Substrate	Y_s	η
Aerobacter aergenes	(17)	Minimal	Glucose	0.145	0.180
"	(17)	Minimal	Mannitol	0.120	0.149
Aerobacter cloucae	(20)	Minimal	Glucose pH = 5.0	0.098	0.122
		"	Glucose pH = 7.2	0.143	0.178
		Complex	Glucose	0.208	0.258
Bifidobacterium bifidum	(28)	Complex	Glucose	0.208	0.258
	(28)	"	Galactose	0.154	0.192
	(28)	"	Mannitol	0.153	0.189
	(28)	"	lactose	0.154	0.191
Clostridium thermo-acetium	(29)	Minimal	Glucose	0.111	0.138
E. coli	(17)	Minimal	Glucose	0.133	0.162
Propionibacterium pentosaceum	(30)	Complex	Glucose	0.208	0.258
Saccharomyces cerevisoae	(30)	Partially defined	Glucose	0.117	0.145
Stoeptococcus faecals	(30)	Partially defined	Glucose	0.122	0.152
Mean				0.154	0.184

Table III. Growth Yields for Mixed Cultures in Minimal Media.

Ref.	Substrate	Y_s	Y_{kcal}	Y_{ave}	η
(14)	glucose	0.454	0.118	3.41	0.564
(33)	glucose	0.411	0.110	3.08	0.510
(33)	sucrose	0.415	0.105	2.96	0.515
(33)	glucose	0.468	0.125	3.51	0.581
(33)	lactose	0.459	0.116	3.27	0.569
(31)	glucose	0.442	---	3.32	0.548
(32)	glucose	0.595	---	4.46	0.738
	Mean	0.485	0.115	3.430	0.580

Table IV. Dependence of Biomass Energetic Yield on Y_{ATP} and $(\alpha+\beta)$.

Y_{ATP}	$\alpha + \beta$	η
28.8	3.167	0.88
28.8	1.167	0.74
10.5	3.167	0.73
10.5	2.167	0.65
10.5	1.167	0.50

Table V: Growth Parameters for Chemostat Cultures of a Number of Microorganisms Grown with Glucose. Reference (39).

Organism	Limiting Factor	m_o	m_e from m_o	m_s	m_e from m_s	Y_0^{max}	from Y_0^{max} η_{max}	Y_s^{max}	from Y_s^{max} η_{max}
A. aerogenes	glucose	1.4	0.04116	0.054	0.04358	44.0	0.65	0.380	0.47
	sulphate	4.9	0.11869	0.2160	0.17433	46.7	0.66	0.23	0.29
	Ammonium	3.7	0.08759	1.080	0.87165	77.8	0.76	0.98	1.10
S. cerevisiae	glucose[(a)]	0.60	0.01453	0.0180	0.01453	34.5	0.59	0.51	0.63
Proteus merabilis	glucose	1.0	0.02421	0	0	51.8	0.68	0.33	0.41
Azotobacter vinelandii	oxygen	5.5	0.13317	0.144	0.11622	13	0.35	0.26	0.32
E. coli	glucose[(a)]	3.0	0.07262	0.090	0.07264	42	0.63	0.53	0.65
	glucose	0.6	0.01453	0.072	0.05811	24.7	0.50	0.35	0.44
	glucose[(b)]	5.1	0.12353	0.558	0.45181	72.6	0.75	0.54	0.66

(a) Values of η_{max} and m_e included in finding average η_{max} and m_e.

(b) + $\overline{C}_{AMP}$

Table VI: Energetic Yield and Result of Consistency Test of the Data of Nishizawa *et al.* (43) where Product Formation Data are reported for Oxygen Limited Growth. Reference (44).

D, hr^{-1}	η	$\eta + \varepsilon + \xi_p$	$y_c + d + z$
0.051	0.496	0.969	0.987
0.097	0.610	1.078	1.048
0.143	0.555	1.010	0.980
0.167	0.638	1.063	1.012

Table VII. Biomass Energetic Yields and Consistency Results for the Growth of Aerobacter aerogenes on Glucose in Glucose Limited Chemostat Experiments with KNO_3 as the Nitrogen Source. Reference (45).

D, hr^{-1}	η	$\eta + \varepsilon + \nu + \xi_p$
0.278	0.438	0.968
0.31	0.497	1.024
0.31	0.442	0.931
0.32	0.436	0.944
0.357	0.496	0.994
0.385	0.468	0.955
0.485	0.453	0.940
0.491	0.472	0.981
0.559	0.459	0.931
0.633	0.447	0.973
0.64	0.431	0.940
0.70	0.438	0.982
0.70	0.403	0.953
0.72	0.456	0.957
0.73	0.460	1.036
	0.453	

Table VIII. Consistency Result of the Data of Brown and Zainudeen (46) for the Growth of Trichoderma reesei on Glucose. Reference (47).

pH	D, hr^{-1}	η	$\eta + \varepsilon$
2.5	0.030	0.57	1.07
3.0	0.037	0.59	1.01
	0.050	0.63	1.06
	0.068	0.62	0.94
	0.080	0.65	0.99
4.0	0.066	0.68	1.02
	0.033	0.68	1.06
	0.110	0.64	0.95

Table IX. Consistency of the Results of Hempfling and Mainzer (42) Using Equation (21); m_{ATP} = 18.9 m Moles ATP/g Dry Wt. Hr. and Y_{ATP}^{max} = 10.3 g Dry Wt./Mole ATP. Reference (12).

Substrate	$\frac{m_{ATP}}{m_0}$	$\frac{Y_0^{max}}{Y_{ATP}^{max}}$
Glucose	21	1.21
Galactose	1.56	1.09
Mannitol	1.07	2.43
Glucose + $\overline{CAMP}$	2.02	2.03

Table X. Calculated and Reported Efficiency of Oxidative Phosphorylation of Aerobacter aerogenes relative to Mitochondrial Efficiency from the Data of J. P. Hadjipetrous et al. (48).[b] Reference (49).

Substrate	η	ε	Efficiency (ϑ) Calculated	Reported
D-Glucose	0.5004	0.1899	0.725	1.033
D-Fructose	0.5239	0.2233	0.683	0.933
Maltose	0.5131	0.2127	0.724	0.967
Sucrose	0.5939	0.2233	0.864	1.067
D-Galactose	0.5059	0.3224	0.495	0.633
D-Mannose	0.4777	0.2951	0.476	0.633

[b] Illustrative Calculation: Consider the organic substrate glucose; $\eta = 0.5004$; $\varepsilon = 0.190$ and $\varepsilon_p = 1 - \eta - \varepsilon = 0.310$. The basis is 1 equivalent of available electrons of organic substrate. Thus $\eta = 0.500$ equivalents of available electrons incorporated into biomass, $\varepsilon = 0.190$ equivalents of avilable electrons transferred to oxygen, and $\varepsilon_p = 0.310$ equivalents of available electrons is in acetate (product). Maximum moles of ATP that can be obtained by oxidative phosphorylation $= A_{max} = \varepsilon * 3/2 = 0.190 * 3/2 = 0.285$. Moles of ATP generated with acetate $= A_p = \varepsilon_p * 0.25 = 0.0775$. Moles of ATP used in forming biomass $= A_b = 12\eta/(\sigma_b Y_b Y_{ATP}) = \frac{12(0.5004)}{(0.462)(4.291)10.3} = 0.3$. Moles of ATP from glycolysis $= A_g = \varepsilon/12 = A_{max}/18 = 0.016$. Moles of ATP actually obtained by oxidative phosphorylation $= A_o = A_b - A_g - A_p = 0.2065$. Efficiency of oxidative phosphorylation $\vartheta = A_o/A_{max} = 0.725$.

CHAPTER III

MICROBIAL GROWTH ON GRAIN DUST IN SHAKE FLASKS

INTRODUCTION

The handling of grain is always accompanied by dust generation (14 - 17). Equipment to collect and control the dust has resulted in the need to identify useful outlets for this by-product. The purpose of this work is to investigate microbial utilization of grain dust as a possible outlet for dust collected at grain handling facilities.

Martin (17) has investigated the physical properties and chemical composition of several different grain dusts. The compositional analysis shows that the dusts contain the same chemical components as whole grains but in different proportions (17). For microbial utilizations of grain dusts, carbohydrates are hydrolyzed to glucose and then used as a source of carbon and energy. Starch is the component of primary interest when grain dust is consumed by microorganisms; however, the protein fraction is important in feeding operations. In most microbial utilization processes, the protein in the grain is used for animal feed. In the process of removing the starch, the percent protein in the residue is increased. The starch in the grain dust may be used to produce single cell protein, ethyl alcohol, acetic acid, pharmaceutical products, or other chemicals of interest. In this work, growth studies were conducted to evaluate the extent and efficiency of grain dust utilization by Candida utilis.

Growth studies on grain dust are of interest because residual sources of carbohydrates are often used for the production of bakers yeast, and for the growth of yeast for use in human foods to improve protein and vitamin content. Single cell protein production for use in animal feedings is also of interest, however, the economics of this are not favorable in many parts of the world. The basic technology for growing yeast on carbohydrates is well established and is commonly used in commercial practice (8). As long as there are protein shortages in many parts of the world, it is natural to expect to see development

work to identify processes for microbial protein production for food and feed uses.

The microbial utilization of by-products and waste materials is expected to grow in importance as supplies of petroleum increase in cost. These waste materials can be classified broadly into two categories (8). These are the solid or semi-solid wastes such as grain dust, low quality grain, spoiled fruit, carob pods, date waste, and molasses, and low concentration wastes such as corn steep water and potato processing waste-water. Examples of growth studies using wastes include the work of Lemmel et al. (12) and Moreton (22) using wastes and wastewater from potato processing, and Paredez-Lopez et al. (23) and Carmargo-Robio et al. (2) who cultivated Candida utilis on juice from nopal fruit.

The microbial utilization of corn, wheat, and milo dusts was investigated in this work. Starch in the dust samples was enzymatically hydrolyzed and used in shake flask experiments as filtered and unfiltered glucose for the cultivation of the yeast Candida utilis. Candida utilis was selected for this work because of its good growth rate, its wide acceptance in previous food and feed applications, and its ability to grow on a wide range of organic molecules. The chemical composition of some of the dust samples and products was also measured. The estimated cost of the microbial protein product is compared to the current selling price of soybean meal, the main competitor.

THEORY

The yield of microbial protein on grain dust may be compared to yields reported in the literature for other organic substrates and to maximum theoretical yields. Both mass yields and energetic yields may be reported. Material and energy balances and regularities associated with growth processes may be used in the analysis of yields (3, 4, 18). The stoichiometric equation for the metabolism of different organic substrates by microorganisms is

$$CH_mO_\ell + aNH_3 + bO_2 \rightarrow y_c CH_pO_nN_q + zCH_rO_sN_t + cH_2O + dCO_2 \qquad (1)$$

where CH_mO_ℓ represents the composition of the organic substrate, $CH_pO_nN_q$ is the composition of these atoms in the biomass, and $CH_rO_sN_t$ is the composition in the extracellular products. Minkevich and Eroshin (18) have found that the weight fraction carbon in biomass, σ_b, the reductance degree, γ_b, and the energy content per equivalent of available electrons in biomass, Q_o, are relatively constant. Kharasch (10) had previously shown that the heat of combustion per equivalent of available electrons is about 26.05 kcal/eq. ave. for a wide variety of organic molecules.

The reductance degree, γ, is the equivalents of available electrons per g atom carbon or the equivalents of oxygen required to oxidize the organic substance of interest to CO_2, H_2O and NH_3.

In Equation (1), y_c is the biomass carbon yield in units of mass of carbon in biomass per mass of carbon in the consumed organic substrate. A more commonly used yield is the mass yield, Y_s, g biomass/g consumed substrate. A third yield is the biomass energetic yield, η, which is the energy content of the biomass per unit of energy in the consumed organic substrate. These yields are related as

$$\eta = y_c \frac{\gamma_b}{\gamma_s} = Y_s \frac{\sigma_b \gamma_b}{\sigma_s \gamma_s} \tag{2}$$

From the first law of thermodynamics, the biomass energetic yield must be less than one; however, based on known biochemistry of microbial metabolism and growth, the biomass energetic yield for aerobic growth must be less than 0.88 (7). When maintenance requirements are considered and the inefficiencies of growth and metabolism are considered actual growth yields are usually much smaller than 0.88.

When the starch in the grain dust is hydrolyzed to glucose and microbial growth occurs with the solid residue from hydrolysis also present in the mixture, yield expressions must be introduced to relate the mixture of undigested grain dust and microbial protein to the quantity of dry dust initially present. The mixture of unutilized grain dust and microbial cells is the product of interest for animal feeding. Let Y_p be the mass of this product per mass of original grain dust on a dry basis. A second measure of the yield is the mass of protein in the final product per mass of original grain dust on a dry basis, Y_{pro}. The protein fraction, x_p, is also of interest in characterizing the protein rich product. If we let x_F be the protein fraction in the dry grain dust, we can relate the mass of protein produced per mass of dust expended, Y_E, to the above yields; that is,

$$Y_E = \frac{Y_{pro} - x_F}{1 - Y_P} \tag{3}$$

Similarly, the energetic yield associated with microbial protein production in terms of energy content of produced protein per unit of energy in the dust evolved as heat, Λ, is given by

$$\Lambda = \frac{(Y_{pro} - x_F)\sigma_{pro}\gamma_{pro}}{\sigma_d \gamma_d - Y_P \sigma_P \gamma_P} \tag{4}$$

where subscript d refers to the original dry dust, subscript p to the product (microbial protein plus residual dust), and subscript pro to protein; σ is weight fraction carbon and γ is the equivalents of avaiable electrons per g atom carbon (see appendix).

MATERIALS AND METHODS

Grain Dust

Corn dust, wheat dust and milo dust were supplied by the U.S. Grain Marketing Research Laboratory, (Manhattan, Kansas). Corn dust I was sample 786-43 which had been obtained from Cargill; Corn dust II was sample 3/23/79 which had been obtained from Cargill; Milo dust I was sample 8/30/78 from Morrison grain elevator; Milo dust II was sample 3/23/79 from Morrison grain elevator; Wheat dust I was sample 8/29/78; and Wheat dust II was sample 1/22/79. The compositions of these grain dusts are presented in Table I.

Organism

Candida utilis Y-1084 obtained from the USDA Northern Regional Research Laboratory was used throughout this experiment.

Medium Composition

An optimum medium composition used by Camargo-Rubio et al. (2) for the growth of Candida utilis on Nopal fruit juice was employed, except that ammonium sulfate $(NH_4)_2SO_4$ was replaced by urea $(NH_2)_2$ in the shake flask experiments to minimize pH problems. The medium composition and trace elements are shown below.

Component	g/liter distilled water
Glucose $C_6H_{12}O_6$ (from dust)	10.00
Urea $(NH_2)_2CO$	1.00
KH_2PO_4	.80
K_2HPO_4	.07
$MgSO_4 \cdot 7H_2O$	.20
$CaCl_2 \cdot 2H_2O$	.05

$ZnSO_4 \cdot 7H_2O$	.02
$MnSO_4 \cdot 4H_2O$	.02
$CuSO_4 \cdot 5H_2O$	.005
$FeSO_4 \cdot 7H_2O$	.10
Na_2SO_4	1.00

Enzymatic Hydrolysis of Grain Dust

Enzymes: Termamyl liquid 60 (60 Kilo Novo units/g), a heat stable α-amylase from Bacillus licheniformis, and amyloglucosidase 150 (150 Novo AG units/ml) from Aspergillus niger were supplied by Novo Laboratories Incorporated, (59 Danbury Road, Wilton, Connecticut 06897).

Hydrolysis: A 50 g sample of the grain dust which had been ground with mortar and pestle was weighed into a flask containing 500 ml of .05 M phosphate buffer at a pH of 5.0. The mixture was stirred thoroughly to ensure that all the dust particles were wet. The mixture was submerged into a constant temperature bath at 60°C and allowed to stand for 24 hours. After the 24 hour period, it was heated over a boiling water bath until a maximum temperature usually about 95°C was reached. This condition was maintained for about 5 minutes with continuous stirring. The grain dust was then autoclaved at 20 psia for 15 minutes to ensure thorough gelatinization of the starch (24). After autoclaving, the mixture was cooled to 100°C and some Termamyl 60 L was added (5 ml/kg starch). The mixture which was continuously stirred was placed in a 60°C water bath for 2 hours to break down the starch to the dextrins. To the "thinned starch" was added Amyloglucosidase 150L (5 g/kg starch). The mixture was held at 60°C for 72 hours. Throughout this period the mixture was constantly stirred. At the end of the hydrolysis the glucose was separated from the solids using

filtration and its quantity was determined analytically. The residue after the hydrolysis was dried at 105°C for 18 hours.

Fermentation

Innoculation: Yeast cells from a YM agar slant culture were incubated for 27 hours at 30°C and innoculated into 250 ml shake flasks containing 100 ml of medium. The cells were cultivated for 18 hours and then used as innoculum for the other flasks.

Growth of Candida utilis on filtered glucose: Ninety ml of culture medium contained in a 250 ml flask was prepared using the glucose filtered after the hydrolysis of the grain dust as substrate. Having adjusted the pH of the medium to 4.5 with the aid of a Horizon pH controller (model 5997), the flask was autoclaved at a steam pressure of 20 psia for 15 minutes. The flask was cooled to 30°C and was innoculated with 10 ml of the innoculum. It was incubated at 30°C in a controlled environment incubator shaker (New Brunswich Scientific Co.) at 250 rpm for 12 hours after which the fermentation was stopped and cell concentration was determined.

Growth of Candida utilis on unfiltered glucose: Ninety ml of culture medium contained in 250 ml flasks were prepared using the filtered glucose. In addition, a calculated amount of the solid residue dried at 105°C for 18 hours after hydrolysis of the starch, was added to give a glucose-solid ratio identical to that of the original grain dust. This was equivalent to hydrolyzing an amount of grain dust containing one gram of glucose and used without filtration. Following the preparation of the culture medium, the pH was again adjusted to 4.5 and the mixture was autoclaved at 20 psia for 15 minutes. The medium was cooled to 30°C, innoculated with 10 ml of the innoculum and incubated at 30°C at 250 rpm for 12 hrs. After the fermentation direct determination

of the cell yield was not possible. However the solid product was centrifuged and dried to constant weight at 105°C. The percentage protein in the dried solid was determined.

Analytical Methods

Glucose concentration: This was assayed by the glucose-DNS method of Miller (20).

Cell dry weight analysis: Yields of cells grown on filtered glucose alone (water soluble substrate) were determined by depositing the cells from a 10 ml suspension on a dried millipore membrane filter (Type RA 1.2 μm). The residue was washed with a second volume of distilled water and then dried at 105°C to a final constant weight.

Nitrogen and protein determination: The percentage nitrogen in the original dry grain dust, residue after hydrolysis, pure biomass, and the product of the fermentation on unfiltered glucose was determined using the micro-Kjeldahl method of Mitchell (21).

The % protein was obtained by multiplying the % nitrogen by 6.25; however, for the case of wheat dust and its products a 5.91 factor was used for the nitrogen originally in the dust and 6.25 was used for the biomass nitrogen.

Elemental composition: The carbon, nitrogen, hydrogen and ash contents of the dried grain dust, residue after hydrolysis, pure biomass, and product of fermentation on unfiltered glucose were measured with a Perkin-Elmer elemental analyzer 240.

RESULTS AND DISCUSSION

Table I shows the results of measuring the protein, moisture, and starch content of the grain dust samples. All samples of grain dust analyzed showed moisture and protein contents which are in good agreement with the values reported by Martin (17). For each of the dust samples, the starch content, as measured using enzymatic hydrolysis, was lower than the value reported by Martin for the corresponding type of grain dust. For the corn dust, enzymatic hydrolysis resulted in 49.2 to 57.1% starch compared to the 60.9 to 67.7% reported by Martin (17). For milo dust, 24.6% starch was obtained using enzymatic hydrolysis compared to 38 to 61.5% reported by Martin. Similarly, wheat dusts analyzed 22.4 to 22.7% starch enzymatically compared to 39.8 to 55.8 as reported by Martin. The differences may be due to the different analytical methods employed. Martin (17) obtained the percent starch by difference. Some components may not be enzymatically hydrolyzed, or if they are, their hydrolysis products are not properly measured by the DNS method. Care was exercised to minimize the problem of incomplete hydrolysis of the grain dust samples by proper pretreatment of the grain dust. Shearing of the dust with mortar and pestle, followed by cooking, and autoclaving at 120°C for 15 min. was carried out to gelatinize the starch prior to adding the Thermamyl at 90-100°C. The dextrinization was then carried out in a 60°C constant temperature bath for 2 hours prior to adding the amyloglucosidase. Madsen et al. (13) recommend thinning at 105-110°C for 5-10 min. followed by dextrinization at 95-100°C for 1 to 2 hrs.

Acid hydrolysis is capable of releasing other products such as galacturonic acid from pectin, hymicellulose, and glycoprotein (22) which will not be enzymatically hydrolyzed by amylases. However, commerically, enzymatic hydrolysis

has several advantages over acid hydrolysis; acid resistant equipment is not required; glucose yields are higher (lower reversion); concentrations of degradation products such as furfurals are lower.

It was noticed that the hydrolysis of wheat starch was more difficult than the other samples since its starch was not easily gelatinized. For the corn dust and milo dust reasonable hydrolysis of the starch was obtained without autoclaving but for the wheat dust little hydrolysis was obtained when the autocalving step was skipped. After hydrolysis, the glucose was separated by filtration. This could be better done by centrifugation when dealing with a large amount of dust since the filtration of the glucose from the unhydrolyzed product through the standard laboratory paper was very slow. It was also observed that the glucose could not adequately be separated by allowing the hydrolysis products to sit. This was because the settling period was too long.

The measured elemental compositions of samples of corn dust, wheat dust, and Candida utilis are reported in Table II. Values for oxygen are obtained by difference. For the corn dust, the reductance degree, $\gamma_d = 4.02$, is close to the value of 4.0 for carbohydrates. The weight fraction carbon, σ_d, is intermediate between 0.44 for starch and 0.4 for glucose. Based on the twenty common amino acids, the arithmetic average values are $\sigma_{pro} = 0.52$ and $\gamma_{pro} = 4.20$ for protein. The measured elemental composition of Candida utilis is in good agreement with that reported by Harrison (9) who found 47% C, 6% H, 9% N, 31% O. The reductance degree, γ_b, is 4.17 compared to 4.05 for the composition reported by Harrison and 4.29 for the average value found by Minkevich et al. (19). The weight fraction carbon is very close to the average value of 0.462 of Minkevich et al. (19).

The elemental compositions of the final products were not measured; they were assumed to be equal to the values for σ_d and γ_d when Equation (4) was used.

The soluble products of grain dust hydrolysis appear to be an excellent medium for growth of Candida utilis. Growth yield values (Y_s) are presented in Table III for growth on glucose from two different dusts, corn dust and milo dust. Average yields are 0.50 and 0.49 g cells/g glucose for glucose from corn and milo dusts, respectively. These values are in good agreement with the value of 0.50 reported by Forage and Righelato (8) and 0.455 reported by Paradez-Lopez et al. (23). Using Equation (2), $\eta = 0.61$ when $Y_s = 0.5$ for growth on glucose; that is 61% of the energy in the glucose is incorporated into cell mass compared to a theoretical maximum of 88%. Because of maintenance requirements and inefficiencies associated with ATP generation and utilization values such as $\eta = 0.61$ which is 69% of 0.88 are to be expected.

The Candida utilis contained 50% crude protein based on the results of Kjeldahl nitrogen measurements and a 6.25 factor to convert nitrogen fraction to protein fraction. Table IV contains the results of the growth of Candida utilis on unfiltered glucose from Corn dust II for several trials. The tables contain the g of protein rich product produced per g of dry corn dust, percent protein in final product, and g of protein obtained in final product per gram of dry corn dust. The table shows that a protein rich product containing about 30% protein could be obtained using corn dust. The results also show that starting with one gram of dry corn dust approximately 0.197 gram of protein could be obtained in the final product. Since the cell yields could not be measured directly, a protein balance on starting dry dust and dry final product indicated a cell yield $Y_s = 0.4787$ g cell/g glucose. The yield, which was based on the quantity of glucose known to be present in the medium, is comparable to

the value obtained for the growth of filtered glucose. The result also shows that for each gram of starting dry grain dust, 0.665 g of final protein rich product were obtained. This ratio is nearly the same as the fraction of available electrons in the original dust retained in the final product. It is also an approximate measure of the energetic efficiency in the conversion of corn dust to protein rich product.

The results for the growth of C. utilis on unfiltered glucose from Milo dust II are shown in Table V. Using milo dust, final products containing about 20% protein were obtained and for each gram of dry dust 0.72 g of dry final product were obtained on the average. The results also show that starting with one gram of dry milo dust, 0.15 g of protein would be obtained in the final product. A protein balance on the original dry dust and final dry product showed that the cell yield is Y_s = 0.450 g cell/g glucose. Contained in Table VI are the results for the growth of Candida utilis on unfiltered glucose from wheat dust II. The results indicate that using wheat dust containing about 22.53% starch, a final product containing 20% protein was obtained. The results also show that about 83% of the mass of the original dry grain dust is recovered in the final product.

Table VII is a summary of the average values reported in Tables IV, V, and VI. The predicted values are shown alongside the experimental values for the g of protein rich product and the g protein in the product based on a gram of dry dust. The predicted values of g protein rich product are the sum of the solid residue after hydrolysis and the estimated cell production based on Y_s = 0.5 and the glucose yield from hydrolysis. The experimental values are expected to be smaller than the predicted values when some of the solid residue is consumed by the cells. These experimental and predicted values and the less than expected yields from starch hydrolysis suggest that glucose adsorbed on

the solid residue or unhydrolyzed starch may be present in the solid fraction and used to support growth. However, other fractions of the solid residue may also be sources of biochemicals used for growth by the cells. For the corn dust, on the average, 66.5% of the original dry mass is in the final product compared to a predicted value of 75.4%. For milo dust, the average predicted value is 86% and the average experimental value is 72%. For wheat dust, the average values are 83% and 88.8% with the predicted value again being larger that the experimental value.

Table VII also contains the experimental and predicted values for the g of protein in the final product per g of dry dust initially present. The predicted values are the sum of the protein present initially and the protein produced by microbial growth on the measured glucose when $Y_s = 0.5$ and the cells are 50% protein. The purpose of comparing experimental and predicted values is to try to identify the extent to which cereal protein is being used as the nitrogen source for microbial protein production. The predicted values exceed the experimental values consistently only in the case of the wheat dust. Some microbial utilization of cereal protein may be present in this case. The two-sided t-test at the 95% significance level was used to determine if the predicted and experimental values differed significantly. For hydrolysis A for the corn dust, for example, a mean of 0.188 and a standard deviation of 3.28% were used with the predicted value of 0.187 in the t-test to show that these two values are not significantly different. Significant differences were found at the 95% confidence level for hydrolysis C with milo dust and for the three wheat dust hydrolyses; however, for hydrolysis C with milo dust the experimental protein content exceeds the predicted while for the wheat dust the predicted exceeds the experimental. Thus, in the wheat dust, wheat protein appears to be utilized to produce microbial protein; however, this does not appear to

happen with corn or milo dust to any significant extent. Thus, based on the protein in the final product, microbial growth without separation of the glucose from the solid residue may be desirable for animal feed production. For animal feeding of the product, the relative costs of processing alternatives should be considered because the additional solid residue will probably influence the heat and mass transfer rates and the productivity of the fermentor.

If grain dust is to be used to produce yeast for food uses, the glucose should be separated from the solid residue prior to fermentation. The solids may be used in animal feeding while the yeast may be used for its protein content and to supply B group vitamins.

Equation (3) may be used to calculate the mass of protein produced per mass of grain dust expended. For corn dust, the average value is 0.35 g protein produced/g dust expended while for milo and wheat dusts the average values are 0.18 and 0.20 g protein/g dust expended, respectively. Equation (4) may be used to estimate the energy in the produced protein per unit of energy evolved as heat or the equivalents of available electrons in the produced protein per equivalent of available electrons in the expended grain dust. Average values are 0.48 for corn dust, 0.23 for milo dust, and 0.26 for wheat dusts. These results show that the best results are obtained for corn dust. Corn dust has the highest starch content and the resulting protein fraction is largest for corn dust.

The processes envisioned for commercially processing grain dust using microorganisms are not significantly different than those envisioned for whole grain. Since the starch is the most important raw material for microbial conversion, the starch content of the dust will influence the economic value of the dust for microbial processes. Since the protein in the dust is usually

recovered and used, it also has an economic value associated with it; however, the moisture, ash, and cellulosic fractions have little economic value. Economic evaluation of the process indicates that grain dust with 60% starch, 9.5% protein, and 14% moisture and valued at 4.17 ¢/lb could be converted to a 33% protein product which could be produced for about 10 ¢/lb. At 2.5 ¢/lb, the 33% protein product could be produced for about 8.4 ¢/lb.

Ethyl alcohol could also be produced using grain dust as the source of glocose for conversion to ethanol. The starch content of the dust will again influence the economic value of the dust for this purpose. Low quality grain is frequently used for production of ethyl alcohol, and no processing difficulties are envisioned if grain dust is used for ethanol production. However, because of the increased ash content and lower starch content of most grain dusts, their economic value will be less than the value of the whole grain.

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Table I. The composition of the grain dust samples.

Dust type	% Protein	% Water	% Starch
Corn dust I	6.69	13.40	57.12
Corn dust II	7.87	10.25	49.20
Milo dust I	8.70	10.39	24.60
Milo dust II	9.95	10.67	24.54
Wheat dust I	12.35	10.40	22.45
Wheat dust II	12.67	10.26	22.70

Table II. Elemental Composition of dry grain dust and biomass.

Substance	% C	% H	% N	% O
Corn dust I	42.17	6.31	1.07	48.07
Corn dust II	41.68	6.86	1.26	48.02
Wheat dust I	40.88	5.34	2.12	—
Candida utilis	45.88	6.45	8.98	31.05

Average values are presented for the grain dusts.

Table III. Growth yield for Candida utilis grown on glucose obtained by enzymatic hydrolysis of starch in grain dust

	Yield, Y_s, g cells/g glucose	
Trial	Corn dust I	Milo dust I
1	0.457	0.446
2	0.450	0.502
3	0.483	0.470
4	0.483	0.509
5	0.524	0.518
6	0.501	0.505
7	0.511	0.498
8	0.500	0.510
9	0.524	0.501
10	0.514	0.445
11	0.529	0.481
Average	0.498	0.490

Table IV. Product yield and protein content from growth of *Candida utilis* on unfiltered glucose from hydrolysis of Corn dust II for hydrolysis experiments A, B, and C.

g protein rich product / g of dry grain dust			Percent protein in product			g protein/g dust		
A	B	C	A	B	C	A	B	C
0.674	0.546	0.712	31.8	25.3	32.2	0.214	0.138	0.229
0.557	0.683	0.710	28.6	30.1	29.5	0.159	0.206	0.209
0.612	0.719	0.704	25.4	30.2	30.1	0.156	0.217	0.212
0.717	0.594	0.710	32.9	26.7	32.2	0.232	0.158	0.229
0.582	0.603	0.648	25.7	25.6	30.9	0.150	0.155	0.200
0.671	0.589	0.720	25.2	26.4	31.3	0.169	0.156	0.226
0.613	0.709	0.662	27.1	29.8	30.3	0.166	0.211	0.201
0.677	0.736	0.731	35.8	31.4	32.0	0.243	0.231	0.234
0.709	0.693		30.2	30.3		0.214	0.210	
0.577			25.7			0.148		
0.694			30.6			0.212		

Table V. Product yield and protein content from growth of *Candida utilis* on unfiltered glucose from hydrolysis of Milo dust II for hydrolysis experiments A, B, and C.

g protein rich product / g dry grain dust			percent protein in product			g protein/g dust		
A	B	C	A	B	C	A	B	C
0.650	0.707	0.676	19.1	18.1	20.9	0.124	0.128	0.142
0.743	0.777	0.697	22.8	19.7	19.7	0.169	0.153	0.137
0.736	0.697	0.727	19.3	17.9	22.4	0.142	0.124	0.163
0.682	0.755	0.714	19.6	20.7	22.7	0.134	0.156	0.163
0.755	0.699	0.756	22.1	18.9	23.1	0.167	0.132	0.174
0.675	0.772	0.735	18.9	20.4	22.7	0.128	0.157	0.167
0.745		0.740	23.4		21.9	0.174		0.163
0.698		0.729	22.8		22.5	0.159		0.164
0.627		0.740	18.5		23.0	0.116		0.170
0.713		0.729	21.4		22.9	0.152		0.167

Table VI. Product yield and protein content from growth of Candida utilis on unfiltered glucose from hydrolysis of Wheat dust II for hydrolysis experiments A, B, and C.

g protein rich product / g dry grain dust			Percent protein in product			g protein/g dust		
A	B	C	A	B	C	A	B	C
0.793	0.830	0.838	19.4	19.9	19.9	0.154	0.165	0.166
0.822	0.869	0.836	19.3	19.7	19.5	0.159	0.171	0.163
0.824	0.855	0.850	19.4	19.9	19.9	0.160	0.170	0.169
0.835	0.847	0.860	21.0	20.3	19.8	0.175	0.172	0.170
0.825	0.790	0.857	20.1	19.9	19.8	0.166	0.157	0.170
0.811	0.829	0.808	19.2	19.9	20.0	0.156	0.165	0.162
0.799	0.840	0.839	20.7	19.8	19.8	0.165	0.167	0.167
0.829	0.795	0.841	20.8	20.5	19.9	0.172	0.163	0.167
0.837	0.791		20.2	20.5		0.169	0.162	
	0.822			20.3			0.167	

Table VII. Average yield values from each series of experiments for Candida utilis grown on grain dust following enzymatic hydrolysis.

Sample	g protein rich product / g dry grain dust		g protein in product / g dry grain dust		Percent protein in final product	g protein produced / g dust expended	available electrons of protein produced per available electron of dust expended
	Experimental value	Predicted value	Experimental value	Predicted value			
Corn dust II							
A	0.644	0.742	0.188	0.187	29.0	0.306	0.474
B	0.652	0.777	0.185	0.199	28.4	0.282	0.363
C	0.700	0.742	0.217	0.208	31.1	0.461	0.596
Milo dust II							
A	0.702	0.864	0.146	0.161	20.8	0.158	0.203
B	0.734	0.896	0.142	0.152	19.3	0.159	0.205
C	0.724	0.820	0.161	0.140	22.2	0.223	0.288
Wheat dust II							
A	0.819	0.889	0.164	0.188	20.0	0.180	0.232
B	0.827	0.886	0.166	0.189	20.0	0.200	0.258
C	0.841	0.889	0.167	0.186	19.8	0.224	0.287

APPENDIX

The weight fraction of carbon and the equivalents of available electrons per g atom carbon are denoted by σ_{pro} and γ_{pro}, respectively. Table 1 contains the reductance degree and carbon weight fraction of amino acids. Based on the twenty common amino acids which make up protein, the arithmetic average values are $\sigma_{pro} = 0.52$ and $\gamma_{pro} = 4.20$ for protein. The value of σ_{pro} is obtained using the carbon weight fractions for the individual amino acids as they appear in protein.

TABLE 1. Reductance degree and carbon weight fraction of amino acids.

Amino Acid	No. of Carbon atoms	Reductance degree, γ	Carbon weight fraction, σ	
			Amino Acid	in protein
1. Alanine	3	4.0	.404	.507
2. Arginine	6	3.67	.414	.462
3. Asparagine	4	3.0	.364	.421
4. Aspartic acid	4	3.0	.361	.417
5. Cysteine	3	3.33	.298	.350
6. Glutamic Acid	5	3.60	.408	.465
7. Glutamine	5	3.60	.411	.469
8. Glycine	2	3.0	.320	.421
9. Histidine	6	3.33	.465	.526
10. Isoleucine	6	5.0	.550	.637
11. Leucine	6	5.0	.550	.637
12. Lysine	6	4.67	.493	.563
13. Methionine	5	4.4	.403	.458
14. Phenylalanine	9	4.4	.655	.735
15. Proline	5	4.4	.522	.619
16. Serine	3	3.33	.343	.414
17. Threonine	4	4.0	.403	.475
18. Tryptophan	11	4.2	.647	.710
19. Tyrosine	9	4.22	.597	.663
20. Valine	5	4.8	.513	.606

CHAPTER IV

MICROBIAL GROWTH ON CORN DUST IN BATCH CULTURE

INTRODUCTION

The handling of grain is always accompanied by dust generation (1 - 4). Equipment to collect and control the dust has resulted in the need to identify useful outlets for this by-product. The purpose of this work is to investigate microbial utilization of grain dust as a possible outlet for dust collected at grain handling facilities.

Martin (4) has investigated the physical properties and chemical composition of several different grain dusts. The compositional analyses show that the dusts contain the same chemical components as whole grains but in different proportions. For microbial utilization of grain dusts carbohydrates are hydrolyzed to glucose and then used as a source of carbon and energy. Starch is the component of primary interest when grain dust is consumed by microorganisms; however, the protein fraction is important in feeding operations. In most microbial utilization processes, the protein in the grain is used for animal feed. In the process of removing the starch the percent protein in the residue is increased. The starch in the grain dust may be used to produce single cell protein, ethyl alcohol, acetic acid, pharmaceutical products, or other chemicals of interest.

Growth studies on grain dust are of interest because residual sources of carbohydrates are often used for the production of bakers yeast, and for the growth of yeast for use in human foods to improve protein and vitamin content. Single cell protein production for use in animal feeding is also of interest; however, the economics of this are not favorable in some parts of the world. The basic technology for growing yeast on carbohydrates is well established and is commonly used in commercial practice (5). As long as there are protein shortages in many parts of the world, it is natural to expect to see development work to identify processes for microbial protein production for food and feed uses.

The microbial utilization of by-products and waste materials is expected to grow in importance as supplies of petroleum increase in cost. These waste materials can be classified broadly into two categories (5). These are the solid or semi-solid wastes such as grain dust, low quality grain, spoiled fruit, carob pods, date waste and molasses, and low concentration wastes such as corn steep water and potato processing waste - water. Examples of growth studies using wastes include the work of Lemmel et al. (6) and Moreton (7) using wastes and wastewater from potato processing and Paredez-Lopez et al. (8) and Carmargo-Rubio et al. (17) who cultivated Candida utilis on juice from napal fruit.

In this work, growth studies were conducted to evaluate the extent and efficiency of grain dust utilization by Candida utilis. Corn dust was selected for this study because it usually contains more starch than dusts from milo and wheat (4). Starch in the dust sample (corn dust) was enzymatically hydrolyzed and used in batch fermentations with and without filtration to remove the residual grain dust solids. Glucose concentration, solid product/biomass concentration, nitrogen concentration, oxygen consumption rate and carbon dioxide evolution rate were recorded and used to check the consistency of the data. The results of aerobic fermentation on both filtered and unfiltered glucose media were used to better understand how and to what extent the solid materials in the unfiltered media were utilized by microorganisms. The results of the experiments were used to estimate the growth yields and some growth related parameters.

For the production of a protein rich animal feed, the extent to which microbial protein is formed from inorganic nitrogen when grain dust protein is already present is of considerable interest.

THEORY

Material and Energy Balance

The yield of microbial protein on grain dust may be compared to yields reported in the literature for other substrates and the maximum theoretical yields. Both mass yields and energetic yields may also be reported. Data collected during fermentation can be analyzed using material and energy balances and regularities associated with growth processes (9, 10, 11). The stoichiometric equation of the metabolism of different organic substrates by microorganisms is

$$CH_mO_\ell + aNH_3 + bO_2 = y_c CH_pO_nN_q + z\,CH_rO_sN_t + cH_2O + dCO_2 \qquad (1)$$

where CH_mO_ℓ, $CH_pO_nN_q$ and $CH_rO_sN_t$ represent the elemental compositions of the organic substrate, biomass and extracellular products respectively. Minkevich and Eroshin (11) have found that the fraction carbon in biomass, σ_b, and the reductance, γ_b, and energy content per equivalent of available electrons in biomass, Q_o, are relatively constant. Kharasch (12) had previously shown that the heat of combustion per equivalent of available electrons is relatively constant for a wide variety of organic molecules. The reductance degree, γ, has been defined as the equivalents of available electrons per g atom carbon or the equivalents of oxygen required to oxidize the organic substance of interest to CO_2, H_2O and NH_3.

A carbon balance on equation (1) yields

$$y_c + z + d = 1 \qquad (2)$$

in which the coefficients y_c, z and d are the fraction of substrate carbon converted to biomass, product and carbon dioxide, respectively.

An available electron balance on equation (1) gives the fractional allocation of available electrons in the organic substrate (13)

$$\frac{4b}{\gamma_s} + y_c \frac{\gamma_b}{\gamma_s} + z \frac{\gamma_p}{\gamma_s} = 1 \tag{3}$$

In the above equation let $4b/\gamma_s$ be denoted by ε, $y_c\gamma_b/\gamma_s$ is denoted by η, and $\frac{z\,\gamma_p}{\gamma_s}$ by ξ_p; they are, respectively, the fractions of available electrons or energy in the substrate, which is evolved as heat, incorporated into biomass and utilized in product formation. Then, equation (3) is reduced to a convenient form as

$$\varepsilon + \eta + \xi_p = 1 \tag{4}$$

During aerobic fermentation of Candida utilis on glucose, product formation can be neglected; that is,

$$y_c + d = 1 \tag{5}$$

$$\varepsilon + \eta = 1 \tag{6}$$

These two equations have been used to check the consistency of data collected during the fermentation of C. utilis on both filtered and unfiltered glucose media. Elemental composition of the strain of C. utilis used in this experiment yielded $\sigma_b = 0.4588$ and $\gamma_b = 4.17$ as compared to the average values of 0.462 and 4.291 reported by Minkevich and Eroshin (11). For consistency checking involving the C. utilis used in this experiment $\sigma_b = 0.4588$ and $\gamma_b = 4.17$ were used.

In equation (1), y_c is the biomass carbon yield in units of mass of carbon in biomass per mass of carbon in the consumed organic substrate. A more commonly used yield is the mass yield, Y_s, g biomass/g consumed substrate. A third yield is the biomass energetic yield, η, which is the energy

content of the biomass per unit of energy in the consumed organic substrate. These yields are related as

$$\eta = y_c \frac{\gamma_b}{\gamma_s} = Y_s \frac{\sigma_b \gamma_b}{\sigma_s \gamma_s} \tag{7}$$

From the first law of thermodynamics, the biomass energetic yield must be less than one, however, based on known biochemistry of microbial metabolism and growth, the biomass energetic yield for aerobic growth must be less than 0.88 (14). Because of maintenance requirements and the inefficiencies of growth and ATP utilization in metabolic processes, actual growth yields are usually much smaller than 0.88.

During fermentation, the measured variables are usually biomass concentration X, g/ℓ, oxygen consumption rate Q_{O_2}, moles O_2/g biomass (hr), carbon dioxide evolution rate Q_{CO_2}, moles CO_2/g biomass (hr), substrate consumption rate, g substrate/g biomass (hr) and nitrogen consumption Q_{N_2}, g nitrogen/g biomass (hr). To estimate values of η, ε, y_c and d relationships between these and the measured variables are employed. In batch cultures two types of balances are possible; they are instantaneous and integrated balances. The following relationships have been derived (13). Using biomass, substrate, O_2 and CO_2 data

$$\eta = \frac{\sigma_b \gamma_b}{\sigma_s \gamma_s} \frac{\mu}{Q_s} \tag{8}$$

$$\varepsilon = \frac{48\, Q_{O_2}}{\sigma_s \gamma_s Q_s} \tag{9}$$

$$y_c = \frac{\sigma_b \mu}{\sigma_s Q_s} \tag{10}$$

$$d = \frac{12 Q_{CO_2}}{\sigma_s Q_s} \tag{11}$$

Methods for obtaining μ, Q_s, Q_{O_2}, and Q_{CO_2} are presented later in this chapter.

For integrated balances the following relationships are used.

$$\eta = \frac{\sigma_b \gamma_b (X-X_o)}{\sigma_s \gamma_s (S_o-S)} \tag{12}$$

$$\varepsilon = \frac{48 \int_0^\theta Q_{O_2} X \, d\theta}{\sigma_s \gamma_s (S_o-S)} \tag{13}$$

$$y_c = \frac{\sigma_b}{\sigma_s} \frac{X-X_o}{(S_o-S)} \tag{14}$$

$$d = \frac{12 \int_0^\theta Q_{CO_2} \, d\theta}{(S_o-S)\sigma_s} \tag{15}$$

where X and S are the concentration of biomass and substrate at any time of interest, respectively, and X_o and S_o are the initial biomass and substrate concentrations. Using substrate and nitrogen data, the biomass energetic yield can be estimated using

$$\eta = \frac{12 \, Q_{N_2} \, \gamma_b}{14 \, q \, \sigma_s \gamma_s Q_s} \tag{16}$$

The value of q in the biomass chemical formula has been found to be about 0.16 (15).

When the starch in grain dust is hydrolyzed to glucose and microbial growth occurs with the solid residue from hydrolysis also present in the mixture, yield expressions must be introduced to relate the mixture of undigested grain dust and microbial protein to the quantity of dry dust initially present. The mixture of utilized grain dust and microbial cells is the product of interest for animal feeding. Let Y_p be the mass of this product per mass of original grain dust on a dry basis. A second measure of the yield is the mass of protein in final product per mass of original

grain dust on a dry basis, Y_{pro}. The protein fraction, x_F, is also of interest in characterizing the protein rich product. If we denote the protein fraction in the dry grain dust by X_F, we can relate the mass of protein produced per mass of dust expended, Y_E, to the above yields; that is

$$Y_E = \frac{Y_{pro} - X_F}{1 - Y_p} \tag{17}$$

Similarly, the energetic yield associated with the microbial protein production in terms of energy content of produced protein per unit of energy in the dust evolved as heat, $\wedge$, is given by

$$\wedge = \frac{(Y_{pro} - X_F)\ \sigma_{pro}\gamma_{pro}}{\sigma_d\gamma_d - \gamma_p\sigma_p Y_p} \tag{18}$$

where subscript d refers to the original dust, subscript p to the product (microbial protein plus residual dust), and subscript pro to protein; σ is weight fraction carbon and γ is the equivalent of available electrons per g atom carbon.

Parameter Estimation

The parameters of interest during microbial growth usually include the maintenance coefficients m_s, m_o, m_D, and m_e which are related to each other according to the equations below (13)

$$m_e = \frac{48 m_o}{\sigma_b \gamma_b} = \frac{12 \gamma_s m_D}{\sigma_b \gamma_b} = \frac{\sigma_s \gamma_s}{\sigma_b \gamma_b} m_s \tag{19}$$

where m_e is the energetic maintenance coefficient, m_o is the oxygen maintanance coefficient, m_D is carbon dioxide maintenance coefficient and m_s is organic substrate maintenance coefficient.

The other set of parameters of interest include the true growth yields Y_s^{max} (substrate consumption for growth), Y_O^{max} (O_2 consumption for growth) and Y_D^{max} (CO_2 evolution due to growth). These true growth yields are also related to the true biomass energetic yield coefficient η_{max} [13].

$$\frac{1}{Y_O^{max}} = \frac{\sigma_b \gamma_b}{48}\left(\frac{1}{\eta_{max}} - 1\right) \tag{20}$$

$$\frac{1}{Y_D^{max}} = \frac{\sigma_b}{12}\left(\frac{\gamma_b}{\gamma_s \eta_{max}} - 1\right) \tag{21}$$

$$Y_s^{max} = \frac{\sigma_s \gamma_s}{\sigma_b \gamma_b} \eta_{max} \tag{22}$$

The parameters can be estimated from the data collected during the fermentation by writing Prit's model in the forms (7).

$$Q_s = \frac{\mu}{Y_s^{max}} + m_s \tag{23}$$

$$Q_{O_2} = \frac{\mu}{Y_o^{max}} + m_o \tag{24}$$

$$Q_{CO_2} = \frac{\mu}{Y_D^{max}} + m_D \tag{25}$$

$$\frac{\mu}{\eta} = \frac{\mu}{\eta_{max}} + m_e \tag{26}$$

MATERIALS AND METHODS

Fermentor

A Bench-Scale Fermentor, Model 19, manufactured by the New Brunswick Scientific Co. was used. The cells were cultivated in a 5 liter stirred container with four vertical baffles which was operated under asceptic conditions. Efficient agitation was achieved by impellers with flat stirring paddles which were magnetically coupled. Filtered air passed through a rotameter and entered the fermentor through a sparger that was located at the bottom of the vessel.

Temperature was maintained at 30°C with the aid of a temperature controller which energizes an in-line immersion heater in the water reservoir or a solenoid value in the cold water inlet line. A thermistor sensing bulb was connected to the controller and a temperature sensing bulb was also connected to a recorder which continuously monitored the temperature of the culture medium. Both of the temperature probes were placed in closed tubes deep in the vessel.

The pH of the medium was maintained automatically at 4.5 by a New Brunswick Scientific Model pH-22 controller which added 2N NaOH, or 2N H_2SO_4 through two peristaltic pumps. The pH of the medium was also continuously recorded.

Dissolved oxygen was continuously measured and recorded using a New Brunswick Scientific Model DO-82 System.

Foaming was controlled both chemically and mechanically. Chemical control was achieved by using Antifoam A spray produced by Dow Corning, and mechanical foam control was realized by means of a foam breaker.

Grain Dust

Corn dust was supplied by the U.S.D.A. Grain Marketing Research Laboratory, Manhattan, Kansas 66502. Corn dust III collected from USDAGMRL was obtained from Cargill. This corn dust was used throughout the experiment.

Organism

Candida utilis Y-1084 obtained from the USDA Northern Regional Research Laboratory was used throughout the experiment.

Medium Composition

An optimum medium composition used by Camargo-Rubio et al. (17) for the growth of Candida utilis on Nopal fruit juice was employed throughout the experiment. The medium composition and trace elements are shown below.

Component	g/liter distilled water
Glucose, $C_6H_{12}O_6$ (from dust)	10.00
$(NH_4)_2SO_4$	2.00
KH_2PO_4	.80
K_2HPO_4	.07
$MgSO_4\ 7H_2O$	.02
$CaCl_2\ 2H_2O$	.05
$ZnSO_4\ 7H_2O$	.02
$MnSO_4\ 4H_2O$	.02
$CuSO_4\ 5H_2O$	.005
$FeSO_4\ 7H_2O$	.10
Na_2SO_4	1.00

Enzymatic Hydrolysis of Grain Dust

Enzymes: Termamyl liquid 60 (60 Kilo Novo units/g) a heat stable α-amylase from Bacillus licheniformis and amyloglucosidase 150 (150 Novo AG units/ml) from Aspergillus niger, were supplied by Novo Laboratories, Incorporated, 59 Danbury Road, Wilton, Connecticut 06897, U.S.A.

Hydrolysis: A 200 g sample of the corn dust III which had been ground with mortar and pestle was weighed into a 5 ℓ flask containing 2 ℓ of 0.05 M phosphate buffer at a pH of 5.0. The mixture was stirred throughly to ensure that all the dust particles were wet. The mixture was submerged into a constant temperature bath at 60°C and allowed to stand for 24 hours. After the 24 hour period, it was heated over a boiling water bath until a maximum temperature of about 95°C was reached. This condition was maintained for about 5 minutes with continuous stirring. The corn dust was then autoclaved at 20 psia for 15 minutes to ensure thorough gelatinization of the starch (Radley 1968). After autoclaving, the mixture was cooled to 100°C and some Termamyl 60 L was added (5 ml/kg starch). The mixture which was continuously stirred was placed in a 60°C water bath for 2 hrs to break down the starch to the dextrins. To the "thinned starch" was added Amyloglucosidase 150 L (5 g/kg starch). The mixture was held at 60°C for 72 hours. Throughout this period the mixture was constantly stirred. At the end of the hydrolysis the glucose was separated from the solids using filtration and its quantity was determined analytically. The residue after the hydrolysis was dried at 105°C for 18 hours.

Fermentation

Inoculation of Fermentor: Yeast cells from a YM agar slant culture were incubated for 24 hours at 30°C and inoculated into 250 ml shake flasks containing 100 ml of medium. The cells were cultivated for 18 hours and then used to inoculate 250 ml medium contained in a 1 liter flask and allowed to grow for

12-14 hours. The 250 ml of cell broth was then transferred into 2.25 ℓ of medium in the fermentor.

Batch Growth of Candida Utilis on Filtered Substrate: 2.25 ℓ of culture medium contained in the 5 ℓ vessel was prepared using the glucose filtered after the hydrolysis of the corn dust as substrate. Having adjusted the pH of the medium to 4.5 with the aid of a Horizon pH Controller (Model 5997), the vessel was autoclaved at a steam pressure of 20 psia for 15 minutes. After cooling, it was connected to the fermentor and inoculated with 250 ml of inoculum after the temperature had been returned to 30°C. During fermentation, air flow rate, glucose concentration, nitrogen concentration, cell concentration, % O_2 in exit air and % CO_2 in exit air were recorded with time. Throughout the fermentation process agitation rate was adjusted to be between 800-1000 rpm.

Batch Growth of Candida Utilis on Unfiltered Substrate: 2.25 ℓ of culture medium was prepared using unfiltered glucose. This was was done by preparing 2.25 ℓ of medium in the 5 ℓ vessel using the glucose filtered after enzymatic hydrolysis of the corn dust as substrate. In addition, for corn dust III which was found to contain 61.8% starch, 15.45 g of the solid residue dried at 105°C for 18 hours after hydrolysis of the starch, was added to give a glucose-solid ratio identical to that of the original grain dust. This was equivalent to hydrolyzing 40.45 gm of corn dust which could be used without filtration. The pH of the mixture was adjusted to 4.5 and then autoclaved at 20 psia for 15 minutes. The medium was allowed to cool before being connected to the fermentor where it was inoculated with 250 ml of the inoculum. During fermentation, air flow rate, glucose concentration, soluble nitrogen concentration, dry weight of product/ℓ, % O_2 in

exit air and % CO_2 in exit air were measured and recorded with time. Also for the entire fermentation period, agitation was maintained between 800 and 1000 rpm.

Sampling: Product samples were taken from the fermentor by means of a sigmamotor peristaltic pump. The first 50 ml were discarded and 20 ml samples were collected and analyzed.

Analytical Methods

Product/Cell Dry Weight Analysis: The 20 ml sample collected each time was filtered on a dried millipore membrane filter (Type RA 1.2 μm). The residue was washed with a second volume of distilled water after the filtrate had been removed. The deposited biomass was dried at 105°C to a final constant weight which was recorded. For the growth of Candida utilis on unfiltered glucose, the residue was the concentration of the dry product which was a combination of cell dry weight and unconsumed solid left after enzymatic hydrolysis.

Glucose Concentration: The glucose obtained in the filtrate of the hydrolysis product and that contained in the filtrate of the samples taken during fermentation were assayed by the glucose-DNS method of Miller (19).

Nitrogen and Protein Determination: The nitrogen concentration in the filtrates of the samples taken during fermentation, and the percentage nitrogen in the original dry corn dust and the products of the fermentation on unfiltered glucose were determined using the micro-Kjeldahl method of Mitchell (20).

The % protein was obtained by multiplying the % nitrogen in the solid samples by 6.25.

Carbon Dioxide and Oxygen Concentrations in the Gas Phase: Throughout the experiment the inlet air was assumed to contain 20.9% O_2 and 0.045% CO_2. The % O_2 and CO_2 in the exit gas stream of the fermentor was continuously monitored by Beckman Model 755 oxygen analyzer and Beckman Model 864 CO_2

infrared analyzer, respectively. The oxygen and carbon dioxide analyzers were arranged in series with Absolute Pressure Control unit made by Beckman Instruments, Inc. The upper and lower scales of the oxygen analyzer were calibrated using dry air and a standard gas containing 17.45% O_2. For the CO_2 analyzer the lower scale was calibrated using dry air while the upper scale was calibrated using a standard gas containing 2.3% CO_2. To remove moisture both sample and standard gases were passed through drierite before entering the pressure regulator. The stream leaving the pressure regulator was passed into the O_2 analyzer and the exit stream on the O_2 analyzer was passed through the pressure regulator from where it was vented into the CO_2 analyzer. By this means the reading obtained from the O_2 analyzer was independent of the variations in barometric pressure in the laboratory.

Volume of Broth: The initial volume of broth was measured and was subsequently corrected as samples were being removed from the fermentor. There were no corrections made for changes that might have been caused by the addition of base or acid, evaporation of water, nutrient consumption and formation of biomass. However for the case of growth on unfiltered glucose corrections were always made for the volume of the solid content due to unhydrolyzed dust.

Air Flow Rate: A rotameter was used to measure the flow rate of the air entering the fermenter. The entering air pressure was recorded using a pressure gauge. The air flow rate was corrected for pressure using a chart provided with the rotameter. The flow rate, F, was measured in ℓ/min and was used to calculate the molar air flow rate per unit volume of broth, Q_m

$$Q_m = \frac{F}{V} \quad \frac{1 \text{ mole gas}}{22.4\ \ell} \cdot \frac{273\ K}{303\ K} \cdot \frac{60 \text{ min}}{\text{hr}} \qquad (27)$$

where V was the volume of broth at the time in question.

The molar flow rate was assumed to be the same for the inlet and exit streams to the fermentor and no corrections were made for dissolved O_2 and CO_2 in the fermentor.

Rate of Oxygen Consumption: Using the value of the molar air flow rate per unit volume, Q_m, the % O_2 in exit stream and the 20.9% O_2 assumed for the inlet air, the rate of oxygen consumption is

$$Q_{O_2} X = \frac{(\%\ O_2 \text{ inlet} - \%\ O_2 \text{ exit})}{100} \quad Q_m \tag{28}$$

From the above oxygen comsumption rate, the specific oxygen consumption rate, Q_{O_2}, was evaluated by the dividing by biomass concentration X (g/ℓ).

Rate of Carbon Dioxide Evolution: Using the value of Q_m calculated, the % CO_2 in exit stream as recorded by the analyzer, and the 0.045% CO_2 in the inlet dry air, the rate of carbon dioxide evolution is

$$Q_{CO_2} = \frac{\%\ CO_2 \text{ exit} - \%\ CO_2 \text{ inlet}}{100} \quad Q_m \tag{29}$$

The specific rate of carbon dioxide evolution Q_{CO_2} was obtained by dividing the above by the biomass concentration, X.

Specific Growth Rate: This is defined as

$$\mu = \frac{1}{X} \frac{dX}{d\theta} \tag{30}$$

It was obtained by finding the slopes of a smooth curve drawn through the biomass concentration-time plots. The slopes were obtained by drawing tangents to the curve at the time in question. By dividing the slope by the value of X at the same point in time, an estimate of the specific growth rate, μ was obtained.

Rate of Glucose Consumption: The specific rate of glucose consumption is defined as

$$Q_s = -\frac{1}{X}\frac{dS}{d\theta} \tag{31}$$

It is also obtained by drawing a smooth curve through the substrate concentration (g/ℓ) vs time (hr) data points and taking slopes at the points in question. The slope is again divided by the value of biomass concentration X at the same point in time.

Rate of Nitrogen Consumption: The specific rate of nitrogen consumption is

$$Q_N = -\frac{1}{X}\frac{dN}{d\theta} \tag{32}$$

Values of the specific rate of nitrogen consumption are obtained graphically from the soluble nitrogen concentrations and biomass concentrations.

RESULTS AND DISCUSSION

Enzymatic hydrolysis of corn dust III showed that it contained about 61.8% starch on a dry weight basis. This value is in good agreement with the 60.9 to 67.7% starch reported by Martin [4].

Elemental analysis of the Candida utilis led to values of $\sigma_b = 0.4588$ and $\gamma_b = 4.17$ which are close to the average values of $\sigma_b = 0.462$ and $\gamma_b = 4.291$ of Minkevich et al. [11]. For application of available electron balances on corn dust, elemental analysis yielded $\sigma_d = 0.420$ and $\gamma_d = 4.02$. The γ_d value is close to the reductance degree value of 4.0 for carbohydrates and the weight fraction carbon σ_d is intermediate between 0.44 for starch and 0.4 for glucose. Based on twenty common amino acids, the arithmetic averages are $\sigma_{pro} = 0.52$ and $\gamma_{pro} = 4.20$ for protein (see Appendix in Chatper III).

Tables I - III contain values of the measured variables from batch fermentation of Candida utilis on filtered glucose obtained by the enzymatic hydrolysis of starch in corn dust III, while the values of the measured variables for the fermentation of the same organism on unfiltered glucose from corn dust III are reported in Tables IV - VI.

Tables VII - XII contain calculated variables from the data in Tables I - VI. These calculated results have been obtained using equations (7-11, 16, 27-32). Generally, the values of the respiratory quotient are less than unity. For the growth of Candida utilis on filtered glucose, respiratory quotients in the range 0.80 - 1.11 were observed during active growing periods of the fermentation. However, in fermentation with unfiltered glucose respiratory quotients in the range 0.61 - 0.95 were obtained during active growth. At low growth rates when metabolism is fully oxidative

Bijkerk and Hall (21) reported a respiratory quotient (of 1.0) for the growth of Saccharomyces ceravisial on glucose. The low values of RQ may be due to product formation or accumulation of dissolved CO_2 and HCO_3^- in the broth which lead to underestimation of carbon dioxide production.

The specific growth rate obtained for fermentation on filtered glucose were generally in the range of 0.20 - 0.60. Unusually high values of μ were obtained at the early stage of Run #2. These anomalously high values were believed to be due to measurement errors in biomass concentration; however, for parameter estimation these high values were deleted. For the fermentation on unfiltered glucose the specific growth rates calculated were in the same range as those obtained for growth on filtered glucose. This suggests that the presence of the unhydrolysed medium does not affect the growth rate of the biomass in the medium.

The biomass energetic yield on an integrated basis is shown in Table XIX. For the growth of Candida utilis on filtered glucose in batch fermentation an integrated average value of η for Runs 1-3 is 0.55 which corresponds to Y_s = 0.454 which is in close agreement with the yield value of 0.5 g cells/g glucose reported for the growth of the same organism in shake flask experiments (22). On the average the value of η obtained for the growth of the organism on unfiltered glucose is 0.59 which corresponds to a Y_s value of 0.49 g cell/g glucose and is comparable to the values reported in the literature (5, 8).

The results of instantaneous consistency tests of the data collected are presented in Tables XIII - XVIII. Using equations (8), (9), (10) and (11), estimates of η, ε, y_c and d were obtained. The consistency of data is based on equations (5) and (6) which give the conditions that $y_c + d = 1$ and $\eta + \varepsilon = 1$ when no extracellular products are produced. For this analysis, an

allowance of deviation from average values may be statistically predicted based on the coefficient of variation of the regularities; that is, the 95% confidence intervals are $0.94 \leq y_c + d \leq 1.06$ and $0.93 \leq \eta + \varepsilon \leq 1.07$. The consistency of data in Runs 1 and 2 is very good with most of the values of $(y_c + d)$ and $(\eta + \varepsilon)$ close to one especially in the intervals where active growth is occuring. However, generally more inconsistent data were seen at the beginning and ends of the fermentation. In some of the analysis where $(\eta + \varepsilon)$ or $(y_c + d)$ is significantly greater than one at the 95% confidence interval, measurement errors are accountable. At the beginning of experiments biomass concentration is prone to error due to its small value; also, the differences in gas composition entering and leaving are small. When values of $(\eta + \varepsilon)$ or $(y_c + d)$ are significantly smaller than unity experimental error or extracellular products could be responsible. Under estimation of CO_2 evolution or O_2 consumption can both lead to values of $(\eta + \varepsilon)$ and $(y_c + d)$ less than unity. Table XV shows that the consistency of data in Run #3 was not very good for growth of unfiltered glucose. For Run #4, values of $(y_c + d)$ were found to be consistent during active growing periods while the values of $(\eta + \varepsilon)$ were greater than unity for Run #4. For Run #5 (Table XVIII) values of $(y_c + d)$ were generally low which can be attributed to low values of d caused by measurement error in CO_2 evolution.

Integrated mass balances were used to obtain the yield and consistency results presented in Table XIX. The results on an integrated basis are quite consistent. The average biomass energetic yield is 0.57. Examination of the results shows that the average values of the respiratory quotient is smaller than expected. The values ranged from 0.82 to 1.02 with mean of 0.86. From these results and the values of $\eta + \varepsilon$ and $y_c + d$, it appears that the measured values of oxygen uptake may be slightly larger and the values of CO_2 evolution slightly smaller than the actual values. It has been shown elsewhere that

some CO_2 which is produced may remain in the liquid phase (21). The consistency of the results appears to be better when integrated balances are used. This is not unusual since tangents must be drawn on the biomass and substrate curves to obtain the instantaneous balances.

Tables XX - XXV contain the results of integrated and instantaneous nitrogen balances and the estimates of biomass energetic yield from the nitrogen data. The values of $(N_0 - N)/(X - X_o)$ and Q_N/μ represent the integrated and instantaneous mass fraction nitrogen in the Candida utilis. The averages of these instantaneous values are presented in Table XXVII and for the growth of Candida utilis on filtered glucose, they range from 0.083 - 0.087. These values are close to the average value of 0.084 reported by Minkevich and Eroshin (11). The instantaneous values were generally higher in the range of 0.085 - 0.102. These values are close to the values of 0.089 reported by Solomon and Erickson (22) for shake flask growth of the same organism. The values of biomass energetic yields calculated using equation (16) were generally of the same magnitude when estimated using biomass - substrate data ($\bar{\eta} = 0.58$) and substrate - nitrogen data ($\bar{\eta}_{N_2} = 0.55$). For growth on unfiltered glucose, the soluble nitrogen instantaneous and integrated balances both gave lower nitrogen composition in biomass than for the corresponding growth on filtered glucose. Also the average estimates of the biomass energetic yields (Table XXVII) were lower when nitrogen data were used as compared to the values obtained using substrate data (Table XIX). The average estimated values of biomass energetic yield for growth or unfiltered glucose is 0.59 using substrate and biomass data while it is 0.48 when obtained from nitrogen data. Table XXVI contained integrated nitrogen balances (g/ℓ) based on nitrogen in products and measured nitrogen concentration in medium. The values obtained using the latter were consistently lower than for the former. On the average total nitrogen used

based on % nitrogen in products is 0.425 (g/ℓ) as compared to 0.379 g/ℓ based on soluble nitrogen concentration in medium.

Table XXVIII contains the values of true biomass energetic yield coefficient, η_{max} and maintenance coefficient, m_e, estimated from pooled data for separate fermentations with filtered glucose and unfiltered glucose. For each set of the pooled data, four different estimates were obtained using substrate consumption Q_s, oxygen consumption, Q_{O_2}, carbon dioxide evolution Q_{CO_2} and biomass energetic yields obtained from nitrogen consumption measurements. Each set of the data was plotted using the SAS procedures (24). Based on both the inconsistency of a data point and a large residual value each of several, outliers was deleted from the data. With the outliers deleted, estimates of η_{max} and m_e were again obtained. These values including the r^2 value for regression analysis are reported in Table XXVIII. In all cases the correlation coefficients are reasonably high and improved after outliers had been deleted from the analysis. For the case of growth on filtered glucose η_{max} ranges from 0.589 to 0.699 and m_e ranges from 0.026 to 0.167 using all of the data; however, without the outliers, η_{max} ranges from 0.575 to 0.652 and m_e ranges from 0.006 to 0.121.

The 95% confidence intervals associated with each point estimate are also presented in Table XXVIII. Note that the confidence interval is reduced in size by rejecting the outliers. The largest confidence interval is associated with the nitrogen data. The smallest confidence intervals are these associated with the oxygen and carbon dioxide measurements. In general, the confidence intervals are supportive; that is, there is an interval from 0.59 to 0.63 for η_{max} which is common to all of the confidence intervals for filtered substrate. Similarly, there is an interval for m_e from 0 to 0.069 which is common to all of the confidence intervals. Using the four different estimates of η_{max} and

m_e from substrate, O_2, CO_2, and nitrogen data with their variance - covariance properties, a single estimate of η_{max} and m_e may be obtained (see Appendix). These combined estimates are $\eta_{max} = 0.619$ and $m_e = 0.043$. The confidence intervals for the individual data sets may be combined using statistical methods (see Appendix). The 90% confidence intervals which result from doing this are shown in Table XXVIII. They are $0.58 \leq \eta_{max} \leq 0.66$ and $0 \leq m_e \leq 0.076$. The combined point estimates and confidence intervals are in good agreement with the average values of $\eta_{max} = 0.65$ and $m_e = 0.03$ which were found from analysis of literature data (see Chapter 2).

For the estimate with outliers included consistent results were obtained using substrate and O_2 data but the η_{max} value obtained from nitrogen - biomass - substrate data was fairly large. However, the confidence interval determination shows that at the 95% confidence interval it is not significantly different from the other estimates.

For the growth on unfiltered substrate, the results of estimating the parameters η_{max} and m_e are also shown in Table XXVIII. Values of the point estimates range from 0.369 to 0.855 for η_{max} and from -0.253 to 0.201 for m_e; however, negative values of m_e are not physically meaningful. In presenting the 95% confidence intervals, only positive values of m_e are considered and η_{max} is assumed to be less than 0.88. The results for unfiltered substrate are not as consistent as those for filtered substrate. The residual grain dust solids, which are present, make it more difficult to obtain accurate measurements of biomass concentration. Assumptions are also involved in both the organic substrate and nitrogen measurements because other sources than the measured values may participate in the growth process. The values of the correlation coefficient, r^2, are somewhat smaller for unfiltered substrate, and the parameter estimation results are much less supportive and less consistent for unfiltered substrate. For the 95% confidence intervals for η_{max} and m_e, there is no

interval common to all four of the data sets for unfiltered substrate. When the four different point estimates and their variance - covariance properties are used to obtain combined point estimates (see Appendix), values of η_{max} = 0.741 and m_e = 0.142 were obtained. From the 97.5% confidence intervals 90% combined intervals for η_{max} could not be found due to the inconsistency of the estimates. For m_e, $0.065 \leq m_e \leq 0.164$ was found (see Appendix). These results are not in good agreement with the results for filtered substrate. The 90% confidence interval for η_{max} for filtered substrate does not include the point estimate for unfiltered substrate. Because the results are not supportive, further experimental work should be conducted in order to obtain accurate estimates of η_{max} and m_e for unfiltered substrate.

For the unfiltered substrate consideration was given to the removal of outliers; however, when the consistency of the data and the size of the residuals was investigated, only one data set was altered by removing outliers (see results for CO_2 and biomass).

In batch fermentation on glucose, growth rates frequently are at or near μ_{max} until the substrate is almost exhausted. Because of this it is difficult to obtain sufficient data at lower growth rates to obtain accurate estimates of the yield and maintenance parameters.

Table XXIX is a summary of product yield and protein content for the growth of Candida utilis on unfiltered glucose. The final products obtained in Runs 4-6 contain on the average 39.73% protein as compared to the 34.36% predicted. The results also show that 58.9% of the mass of the original dry dust is recovered in the product compared to a predicted value of 70.8%. The deviation is due to experimental difficulties. This is because some of the solid dust stick to the wall of the fermentor and makes sampling difficult. This accounts for the low dry weight recovery and higher protein percentage in the final products.

Table XXIX also contains the experimental and predicted values for the g of protein in the final product per g of dry dust initially present. The predicted values are the sum of the protein present initially and the protein produced microbially on the measured glucose when Y_s = 0.5 g cell/g glucose and the cells contain 50% protein. The average predicted value is 0.244 and the average experimental value is 0.230.

Equation (18) may be employed to calculate the mass of protein produced per mass of corn dust expended. These values range from 0.329 to 0.444 with a mean of 0.396. This value is a little bit higher than the value of 0.35 g protein produced per g dust expended obtained on corn dust II in shake flask experiments. However, this is due to the higher starch content of corn dust III (61.8%) as compared to 49.20% in corn dust II. Equation (19) may be used to estimate the energy in the produced protein per unit of energy evolved as heat or the equivalents of available electrons in the protein produced per equivalent of available electrons in the expended dust. For corn dust III the value ranges from 0.426 to 0.574 with a mean of 0.512.

If grain dust is to be utilized to produce protein for food uses, fermentation with filtered substrate would be desirable. However for the production of animal feeds the fermentation may be done on unfiltered substrate. Generally, growth of Candida utilis on unfiltered substrate has no significant advantage over growth on filtered substrate. The low estimates of biomass energetic yield based on nitrogen consumption, indicate that some nitrogen from the original dust solid residue (protein in corn dust) is being used by biomass for growth although not to a highly significant level.

The application of material and energy balances to the fermentation processes gave an insight into the distribution of energy in substrate when it is being used for microbial cultivation. For parameter estimation, data collected

from continuous fermentation or fed-batch fermentation may be more reliable than those obtained from batch processes.

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NOMENCLATURE

a = Moles of ammonia per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon

b = Moles of oxygen per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon

c = Moles of water per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon

d = Moles of carbon dioxide per quantity of organic substrate containing one g atom carbon, g mole/g atom carbon

F = Gas Flow Rate, liters/min

ℓ = Atomic ratio of oxygen to carbon in organic substrate, dimensionless

m = Atomic ratio of hydrogen to carbon in organic substrate, dimensionless

m_D = Rate of carbon dioxide evolution for maintenance, g moles/g dry biomass (hr)

m_e = Rate of organic substrate consumption for maintenance, g equiv. of available electrons in biomass (hr) or kcal/kcal of biomass (hr)

m_o = Rate of oxygen uptake for maintenance, g moles/g dry biomass (hr).

m_s = Rate of organic substrate consumption for maintenance, g/g of dry biomass (hr)

n = Atomic ratio of oxygen to carbon in biomass, dimensionless

P = Atomic ratio of hydrogen to carbon in biomass, dimensionless

Q_{CO_2} = Rate of evolution of carbon dioxide, g moles/g dry wt (hr)

Q_m = Molar air flow rate per unit volume of broth, g mole/ℓ (hr)

Q_N = Specific rate of nitrogen consumption, g/g dry wt (hr)

Q_o = Heat evolution in fermentation per equivalent of oxygen uptake, kcal/g equiv.

Q_s = Rate of organic substrate consumption, g/g dry wt (hr)

q = Atomic ratio of nitrogen to carbon in biomass, dimensionless

r = Atom ratio of hydrogen to carbon in products, dimensionless

S = Organic substrate concentration, g/liter

S_o = Initial organic substrate concentration, g/liter

s = Atomic ratio of oxygen to carbon in products, dimensionless

t = Atomic ratio of nitrogen to carbon in products, dimensionless

X = Biomass concentration, g/liter

X_F = Protein fraction in the dry grain dust, dimensionless

Y_D^{max} = True biomass yield based on CO_2 evolution, g dry biomass/g mole

Y_E = Mass of protein produced per mass of dust expended, dimensionless

Y_O^{max} = True biomass yield based on oxygen, g dry biomass/g mole

Y_{pro} = Mass of protein in final product per mass of original grain dust, dimensionless

Y_P = Mass of protein rich product per mass of original grain dust, dimensionless

Y_s = Biomass yield on organic substrate, g dry wt/g substrate

Y_s^{max} = True biomass yield based on organic substrate, g dry wt/g substrate

y_c = Biomass carbon yield (fraction of organic substrate carbon in biomass), dimensionless

V = Volume of broth, liters

z = Fraction of organic substrate carbon in products, dimensionless

γ_b = Reductance degree of biomass, equivalents of available electrons per g atom carbon.

γ_d = Reductance degree of grain dust, equivalents of available electrons per g atom carbon

γ_p = Reductance degree of products, equivalents of available electrons per g atom carbon

$\hat{\gamma}_p$ = Reductance degree of final protein rich product, equivalents of available electrons per g atom carbon

γ_s = Reductance degree of organic substrate, equivalents of available electrons per g atom carbon

ε = Fraction of energy in organic substrate which is evolved as heat, dimensionless

η = Fraction of energy in organic substrate which is converted to biomass or biomass energetic yield, dimensionless

η_{N_2} = Biomass energetic yield based on nitrogen and substrate data, dimensionless

η_{max} = Maximum biomass energetic yield, dimensionless

μ = Specific growth rate, hr^{-1}

ξ_p = Fraction of energy in organic substrate which is converted to products, dimensionless

σ_b = Weight fraction carbon in biomass, dimensionless

σ_d = Weight fraction carbon in grain dust, dimensionless

$\hat{\sigma}_p$ = Weight fraction carbon in final protein rich product, dimensionless

σ_{pro} = Weight fraction carbon in protein, dimensionless

σ_s = Weight fraction carbon in organic substrate, dimensionless

θ = Time, hrs.

Λ = Energy content of produced protein per unit of energy in the dust evolved as heat, dimensionless

$\underline{\varepsilon}_i$ = Correlated error terms defined in equations (33, 34, 35 and 36)

$\sharp$ = Variance - Covariance structure of the distributions

Table I. Measured variables for batch fermentation of Candida utilis on filtered flucose from corn dust III; Run #1.

θ, hr	volume, ℓ	O_2, %	CO_2, %	X, g/ℓ	S, g/ℓ	N, g/ℓ	F, ℓ/min
0.0	2.500	20.90	0.050	0.640	10.00	0.4354	1.39
1.0	2.445	20.41	0.495	1.063	8.26	0.3878	1.39
2.0	2.369	20.0	0.782	1.444	7.49	0.3673	1.39
3.0	2.296	19.72	1.080	1.888	6.56	0.3265	1.39
4.0	2.219	19.30	1.550	2.616	4.68	0.2925	1.39
4.5	2.107	18.97	1.825	3.431	4.55	0.2449	1.39
5.0	2.035	18.75	2.025	3.820	3.18	0.2211	1.39
5.5	1.950	18.45	2.250	4.359	1.53	0.1524	1.39
6.0	1.867	18.24	2.500	5.134	0.23	0.0884	1.40
6.5	1.782	19.55	1.237	5.284	0.007	0.0246	1.96
7.0	1.702	20.05	0.600	5.640	0	0.0245	1.95
8.0	1.541	20.6	0.357	5.559	0	0.0197	1.41
8.5	1.463	20.7	0.247	5.284	0	0.0197	1.41
9.5	1.375	20.75	0.175	5.493	0	0.0197	1.40
10.0	1.282	20.7	0.182	5.525	0	0.0197	1.11
10.5	1.191	20.7	0.175	5.540	0	0.0197	1.11

Table II. Measured variable for batch fermentation of Candida utilis on filtered glucose from corn dust III; Run #2.

θ, hr	volume, ℓ	O_2, %	CO_2, %	X, g/ℓ	S, g/ℓ	N, g/ℓ	F, ℓ/min
0.0	2.846	20.90	0.050	0.031	14.99	0.4354	1.41
1.0	2.421	20.47	0.475	0.155	14.32	0.4082	1.39
2.0	2.343	20.20	0.638	0.309	14.05	0.4218	1.39
3.0	2.274	20.04	0.900	0.514	13.51	0.3878	1.38
4.0	2.191	19.56	1.113	0.924	13.38	0.3401	1.40
4.5	2.109	18.99	1.650	1.398	11.37	0.2993	1.39
5.0	2.035	18.65	1.925	2.027	10.19	0.2653	1.38
5.5	1.964	18.35	2.300	2.469	9.58	0.2177	1.40
6.0	1.885	18.16	2.488	3.225	8.03	0.1568	1.39
6.3	1.768	18.14	2.500	3.882	6.58	0.1048	1.40
6.5	1.703	18.80	1.980	4.184	5.49	0.0728	1.95
7.0	1.600	18.96	1.950	4.960	3.47	0.0150	1.95
8.0	1.466	19.35	1.500	6.855	0.35	0.0095	1.95
9.0	1.394	20.10	0.675	6.884	0.09	0.0095	1.95
10.0	1.292	20.40	0.313	7.715	0	0.0095	1.95

Table III. Measured variables for batch fermentation of Candida utilis on filtered glucose from corn dust III; Run #3.

Θ, hr	volume,	O_2, %	CO_2, %	X, g/ℓ	S, g/ℓ	N, g/ℓ	F, ℓ/min
0.0	2.474	20.90	0.050	0.852	6.855	0.4354	1.390
1.0	2.375	20.60	0.383	1.031	6.615	0.4095	1.357
2.0	2.307	20.40	0.525	1.186	6.341	0.4082	1.357
3.0	2.225	20.15	0.725	0.986	5.429	0.3837	1.360
4.0	2.142	19.78	1.125	1.623	4.113	0.3435	1.357
5.0	2.068	19.50	1.488	2.558	2.091	0.2626	1.360
5.5	2.002	19.35	1.700	3.204	0.891	0.2177	1.370
6.0	1.914	19.15	1.935	3.814	0.254	0.1497	1.372
6.5	1.839	19.89	1.200	3.848	0.089	0.1143	1.372
7.5	1.763	20.62	0.275	3.897	0	0.1122	1.372
8.0	1.670	20.70	0.200	3.944	0	0.0986	1.350
8.5	1.570	20.78	0.155	3.581	0	0.1054	1.370
9.0	1.460	20.78	0.150	4.212	0	0.1020	1.553

Table IV. Measured variables in fermentation of Candida utilis on unfiltered glucose from corn dust III; Run #4.

θ, hr	volume, ℓ	O_2, %	CO_2, %	dry wt/ℓ	S, g/ℓ	N, g/ℓ	F, ℓ/min
0.0	2.500	20.90	0.05	4.475	9.597	0.449	1.360
1.0	2.456	20.70	0.36	4.485	8.774	0.429	1.357
2.0	2.365	20.37	0.46	4.777	8.089	0.405	1.357
2.87	2.275	20.15	0.51	5.327	7.678	0.384	1.357
4.0	2.215	19.56	0.96	4.670	6.170	0.320	1.380
5.0	2.142	19.15	1.30	6.341	5.758	0.310	1.372
6.0	1.986	18.77	1.83	7.865	3.839	0.228	1.360
6.5	1.914	18.75	1.90	8.216	2.296	0.177	1.370
7.0	1.844	18.42	2.26	8.842	1.117	0.140	1.372
7.5	1.771	18.87	1.90	9.627	0	0.070	1.390
8.5	1.692	20.00	0.54	9.482	0	0.052	1.390
9.5	1.627	20.20	0.29	9.404	0	0.058	1.390
10.0	1.557	20.30	0.19	9.397	0	0.057	1.390

Final dry weight concentration was 9.337 g/ℓ based on a larger sample.

Table V. Measured variables in batch fermentation of C. utilis on unfiltered glucose from corn dust III: Run #5.

θ, hr	volume, ℓ	O_2, %	CO_2, %	S, g/ℓ	dry wt/ℓ	N, g/ℓ	F, ℓ/min
0.0	2.350	-	-	12.600	5.115	0.404	1.370
1.0	2.280	20.55	0.400	12.339	5.148	0.401	1.370
2.0	2.201	20.28	0.550	10.968	5.248	0.393	1.390
3.0	2.126	19.93	0.800	10.694	5.730	0.378	1.373
4.0	2.042	19.53	1.050	9.460	6.463	0.347	1.395
5.0	1.962	18.95	1.537	7.541	7.303	0.272	1.390
5.5	1.871	18.60	2.025	6.032	7.624	0.245	1.375
6.0	1.792	18.44	2.225	5.210	8.893	0.197	1.375
6.5	1.701	18.20	2.500	2.948	9.726	0.146	1.360
7.0	1.631	18.80	2.025	0.411	10.460	0.062	1.900
7.5	1.544	19.80	1.090	0.151	10.882	0.031	1.900
8.0	1.448	20.18	0.600	0.062	11.220	0.025	1.890

Final dry weight concentration was 10.61 g/ℓ based on a larger sample.

Table VI. Measured variable for fermentation of C. utilis on unfiltered glucose from corn dust III; Run #6.

θ,hr	V,ℓ	O_2,%	CO_2,%	X,g/ℓ	S,g/ℓ	N,g/ℓ	F,ℓ/min
0.0	2.500	--	--	5.125	11.707	0.3980	--
1.0	2.434	20.53	0.410	5.237	11.296	0.3880	1.36
2.0	2.364	20.25	0.575	5.315	11.101	0.3814	1.357
3.0	2.259	19.95	0.773	5.396	10.210	0.3742	1.357
4.167	2.185	19.38	1.093	6.023	7.453	0.2978	1.36
5.0	2.105	18.95	1.720	6.939	5.985	0.2473	1.38
5.5	2.027	18.86	1.825	7.872	3.839	0.1961	1.38
6.0	1.953	18.40	2.288	9.130	1.645	0.1412	1.373
6.5	1.883	18.93	1.900	9.800	0.153	0.0816	1.90
7.0	1.824	19.10	1.513	10.595	0.122	0.0318	1.65
8.0	1.751	19.97	0.613	11.073	0.045	0.0318	1.63
9.0	1.670	20.46	0.350	10.649	0	0.0318	1.37

Final dry weight concentration was 10.449 g/ℓ based on a large sample.

Table VII. Calculated Variables in batch fermentation of Candida utilis on filtered glucose from corn dust III; Run #1.

θ,hr	μ,hr^{-1}	Q_{O_2},$\frac{mmoles}{hr.\ g}$	Q_{CO_2}, $\frac{mmoles}{g.\ hr}$	Q_s,$\frac{g}{g.\ hr}$	R.Q
0	--	--	--	--	--
1.0	0.403	6.72	6.10	0.591	0.908
2.0	0.357	9.10	7.40	0.580	0.814
3.0	0.331	8.57	7.48	0.611	0.873
4.0	0.301	8.56	8.02	0.602	0.937
4.5	0.303	9.07	8.47	0.606	0.920
5.0	0.280	9.32	8.56	0.592	0.919
5.5	0.274	9.68	8.89	0.644	0.918
6.0	0.203	9.93	9.01	0.914	0.907
6.5	0.111	6.80	5.98	--	0.880
7.0	--	4.16	2.68	--	0.647
8.0	--	1.19	1.22	--	1.03
8.5	--	0.89	0.88	--	0.987
9.5	--	0.67	0.56	--	0.833
10	--	0.76	0.50	--	0.658
10.5	--	0.81	0.51	--	0.630

Table VIII. Calculated variables in batch fermentation of Candida utilis on filtered glucose from corn dust III; Run #2.

θ, hr	μ, hr^{-1}	Q_{O_2}, $\frac{mmoles}{hr.g}$	Q_{CO_2}, $\frac{mmoles}{hr.g}$	Q_s, $\frac{g}{hr.g}$	R.Q
0.0	-	-	-	-	-
1.0	1.30	46.79	46.25	2.97	1.00
2.0	0.75	32.45	27.24	2.25	0.84
3.0	0.73	19.36	19.14	1.48	0.99
4.0	0.60	15.24	12.12	1.18	0.80
4.5	0.54	18.40	15.41	1.12	0.84
5.0	0.42	17.11	14.26	0.98	0.83
5.5	0.49	17.76	15.67	0.99	0.88
6.0	0.48	15.11	13.44	0.88	0.89
6.3	0.43	13.87	12.31	1.14	0.89
6.5	0.45	13.93	12.80	1.12	0.92
7.0	0.34	11.72	11.49	0.960	0.98
8.0	0.18	9.20	6.88	0.44	0.75
9.0	-	5.46	4.28	-	0.78
10.0	-	3.583	1.927	-	0.538

Table IX. Calculated variables in batch fermentation of C. utilis on filtered glucose from corn dust III; Run #3.

θ, hr	μ, hr^{-1}	Q_{O_2}, $\frac{mmoles}{hr.g}$	Q_{CO_2}, $\frac{mmoles}{hr.g}$	Q_s, $\frac{g}{hr.g}$	R.Q
0.0	-	-	-	-	-
1.0	0.170	4.59	5.10	0.41	1.11
2.0	0.240	6.45	6.12	0.52	0.95
3.0	0.301	7.49	6.69	0.65	0.89
4.0	0.304	8.82	8.32	0.75	0.94
5.0	0.320	8.15	8.37	0.73	1.03
5.5	0.290	8.01	8.52	0.57	1.06
6.0	0.250	8.36	8.90	0.41	1.06
6.5	0.080	4.73	5.38	-	1.14
7.5	-	1.35	1.09	-	0.80
8.0	-	0.99	0.74	-	0.75
8.5	-	0.71	0.62	-	0.87
9.0	-	0.64	0.53	-	0.83

Table X. Calculated values for the batch growth of Candida utilis on unfiltered glucose from hydrodysis of corn dust III; Run #4.

θ,hr	$\frac{dX}{d\theta}$, g/ℓ.hr	$-\frac{dS}{d\theta}$, $\frac{g}{hr.\ell}$	Q_m, $\frac{mmoles}{hr.\ell}$	XQ_{O_2}, $\frac{mmoles}{hr.\ell}$	XQ_{CO_2}, $\frac{mmoles}{hr.\ell}$	R.Q.
1.0	0.200	.512	1333.32	2.67	4.13	1.55
2.0	0.353	.759	1383.80	7.33	5.67	0.77
3.0	0.529	.967	1438.55	10.790	6.62	0.61
4.0	0.609	1.174	1502.56	20.	13.67	0.68
5.0	0.778	1.545	1542.31	27.00	19.29	0.71
5.5	0.875	1.667	1597.77	31.372	25.23	0.80
6.0	1.00	1.938	1651.84	35.20	29.42	0.84
6.5	1.056	2.154	1726.45	40.75	31.95	0.78
7.0	1.059	2.417	1778.92	44.13	39.32	0.89
7.5	-	-	1893.73	38.44	35.03	0.91
8.5	-	-	1981.36	17.44	9.71	0.56
9.5	-	-	2060.98	14.42	4.94	0.34
10.0	-	-	2152.48	12.91	3.01	0.23

Table XI. Calculated variables in batch fermentation of C. utilis on unfiltered glucose from corn dust III; Run #5.

θ, hr	$\frac{dX}{d\theta}$, $\frac{g}{\ell.hr}$	$-\frac{dS}{d\theta}$, $\frac{g}{\ell.hr}$	Q_m, $\frac{mmoles}{hr.\ell}$	XQ_{O_2}, $\frac{mmoles}{hr.g}$	XQ_{CO_2}, $\frac{mmoles}{hr.g}$	R.Q
1.0	0.111	0.50	1450.16	5.08	5.15	1.01
2.0	0.278	0.89	1524.2	9.45	7.70	0.81
3.0	0.548	1.10	1560.61	15.14	11.78	0.78
4.0	0.800	1.62	1648.87	22.59	16.57	0.73
5.0	1.10	2.30	1709.96	33.34	25.52	0.73
5.5	1.25	3.14	1780.15	40.94	35.25	0.86
6.0	1.563	3.43	1858.72	45.72	40.52	0.89
6.5	1.733	4.00	1928.69	52.10	47.37	0.91
7.0	1.00	-	2817.52	59.17	55.79	0.94
7.5	-	-	2965.86	32.62	30.99	0.95
8.0	-	-	3149.70	22.68	17.48	0.77

Table XII. Calculated variables in batch fermentation of C. utilis on unfiltered glucose from corn dust III; Run #6.

θ,hr	$\frac{dX}{d\theta}$, $\frac{g}{hr.\ell}$	$-\frac{dS}{d\theta}$, $\frac{g}{hr.\ell}$	Q_m, $\frac{mmoles}{hr.\ell}$	XQ_{O_2}, $\frac{mmoles}{hr.g}$	XQ_{CO_2}, $\frac{mmoles}{hr.g}$	$-\frac{dN}{d\theta}$, $\frac{g}{hr.\ell}$	R.Q
1.0	0.171	0.360	1348.69	4.99	4.923	0.016	0.99
2.0	0.294	0.600	1385.57	9.01	7.344	0.024	0.82
3.0	0.467	1.220	1447.55	13.75	10.53	0.042	0.77
4.0	0.792	2.170	1502.51	22.84	15.74	0.063	0.69
5.0	1.318	2.92	1582.56	30.86	26.51	0.082	0.86
5.5	1.667	3.33	1642.88	33.51	29.24	0.092	0.87
6.0	2.282	3.733	1676.78	41.92	37.60	0.100	0.90
6.5	-	-	2431.16	47.89	45.098	-	0.94
7.0	-	-	2183.63	39.31	32.04	-	0.82
8.0	-	-	2248.97	20.92	12.76	-	0.61
9.0	-	-	1980.48	8.714	6.04	-	0.69

Table XIII. Examination of data consistency using instantaneous available electron and carbon, balances for growth of C. utilis on filtered glucose from corn dust III; Run #1.

θ, hr	η	ε	y_c	d	$\eta + \varepsilon$	$y_c + d$
0.0	-	-	-	-	-	-
1.0	0.820	0.340	0.780	0.210	1.16	0.990
2.0	0.740	0.470	0.710	0.38	1.21	1.09
3.0	0.648	0.415	0.620	0.37	1.06	0.990
4.0	0.600	0.430	0.570	0.40	1.03	0.970
4.5	0.600	0.420	0.570	0.420	1.02	0.990
5.0	0.560	0.470	0.540	0.430	1.03	0.970
5.5	0.510	0.450	0.490	0.420	0.960	0.910
6.0	0.350	0.330	-	-	0.680	-

Table XIV. Examination of consistency using instantaneous available electron and carbon balances for growth of C. utilis on filtered glucose from corn dust III; Run #2.

θ, hr	η	ε	y_c	d	$\eta + \varepsilon$	$y_c + d$
0.0	-	-	-	-	-	-
1.0	0.52	0.47	0.50	0.47	0.99	0.97
2.0	0.36	0.43	0.34	0.36	0.79	0.70
3.0	0.59	0.39	0.57	0.39	0.98	0.95
4.0	0.61	0.39	0.58	0.35	1.00	0.93
4.5	0.58	0.49	0.55	0.41	1.07	0.96
5.0	0.51	0.53	0.49	0.44	1.04	0.93
5.5	0.52	0.48	0.52	0.44	1.00	0.96
6.0	0.58	0.46	0.56	0.41	1.04	0.97
6.3	0.45	0.37	0.44	0.32	0.82	0.76
6.5	0.47	0.38	0.47	0.34	0.85	0.81
7.0	0.45	0.39	0.43	0.36	0.84	0.79
8.0	0.49	0.63	0.47	0.47	1.12	0.94

Table XV. Examination of consistency of data in batch fermentation of Candida utilis on filtered glucose from corn dust III; Run #3.

θ, hr	η	ε	y_c	d	$y_c + d$	$\eta + \varepsilon$
1.0	0.47	0.34	0.48	0.38	0.86	0.81
2.0	0.56	0.38	0.54	0.36	0.90	0.94
3.0	0.55	0.35	0.53	0.31	0.84	0.90
4.0	0.49	0.35	0.47	0.39	0.86	0.84
5.0	0.52	0.33	0.50	0.34	0.84	0.85
5.5	0.51	0.42	0.49	0.45	0.94	0.93
6.0	0.74	0.61	0.71	0.65	1.36	1.35

Table XVI. Examination of consistency of data in batch fermentation of Candida utilis on unfiltered glucose from corn dust III; Run #4.

θ, hr	η	ε	y_c	d	$y_c + d$	$\eta + \varepsilon$
1.0	0.47	0.16	0.45	0.24	0.69	0.630
2.0	0.56	0.29	0.53	0.23	0.76	0.85
3.0	0.65	0.33	0.63	0.21	0.84	0.98
4.0	0.62	0.52	0.60	0.35	0.95	1.14
5.0	0.59	0.52	0.57	0.38	0.95	1.10
5.5	0.63	0.57	0.60	0.45	1.05	1.20
6.0	0.62	0.54	0.59	0.47	1.06	1.16
6.5	0.59	0.57	0.56	0.44	1.00	1.16
7.0	0.52	0.55	0.50	0.49	0.99	1.07

Table XVII. Examination of consistency of data in batch fermentation of Candida utilis on unfiltered glucose from corn dust III; Run #5.

θ, hr	η	ε	y_c	d	$y_c + d$	η + ε
1.0	0.270	0.300	0.260	0.310	0.570	0.57
2.0	0.370	0.320	0.360	0.260	0.620	0.69
3.0	0.590	0.410	0.570	0.320	0.89	1.00
4.0	0.590	0.420	0.570	0.310	0.88	1.01
5.0	0.570	0.430	0.550	0.330	0.88	1.00
5.5	0.480	0.390	0.460	0.340	0.80	0.87
6.0	0.540	0.400	0.520	0.360	0.88	0.94
6.5	0.520	0.390	0.500	0.360	0.86	0.91

Table XVIII. Examination of consistency of data in batch fermentation of *C. utilis* on unfiltered glucose from corn dust III; Run #6.

θ, (hr)	η	ϵ	y_c	d	$y_c + d$	$\eta + \epsilon$
1.0	0.57	0.42	0.55	0.42	0.97	0.99
2.0	0.59	0.46	0.57	0.38	0.95	1.05
3.0	0.46	0.34	0.44	0.26	0.70	0.80
4.0	0.44	0.32	0.42	0.22	0.64	0.76
5.0	0.54	0.32	0.52	0.28	0.80	0.86
5.5	0.60	0.30	0.58	0.26	0.84	0.90
6.0	0.73	0.34	0.70	0.30	1.00	1.07

Table XIX. Examination of data consistency using integrated available electron and carbon balances for growth of C. utilis on glucose from corn dust III.

Source of data	Time interval (hr)	η	ε	$\eta + \varepsilon$	y_c	d	$y_c + d$
Run #1	0-6.5	0.60	0.47	1.07	0.57	0.41	0.98
Run #2	0-8	0.55	0.46	1.01	0.53	0.41	0.94
Run #3	0-7.5	0.53	0.44	0.97	0.51	0.45	0.96
Run #4[a]	0-6.5	0.61	0.43	1.04	0.59	0.33	0.92
Run #5[a]	0-8	0.58	0.47	1.05	0.56	0.41	0.97
Run #6[a]	0-8	0.61	0.45	1.06	0.58	0.37	0.95

a - Experiment was conducted on unfiltered glucose.

Table XX. Weight fraction nitrogen in biomass from integrated and instantaneous balances and instantaneous biomass energetic yield based on nitrogen and organic substrate measurements for batch fermentation of Candida utilis on filtered glucose from corn dust III; Run #1.

Θ, hr	$\frac{N_o - N}{X - X_o}$ $\frac{g}{g}$	$\frac{Q_N}{\mu}$, $\frac{g}{g}$	$\frac{Q_N}{Q_s}$, $\frac{g}{g}$	η_{N_2}
1.0	0.098	0.073	0.050	0.695
2.0	0.086	0.083	0.051	0.711
3.0	0.085	0.075	0.041	0.571
4.0	0.081	0.075	0.037	0.520
4.5	0.074	0.074	0.037	0.514
5.0	0.075	0.089	0.042	0.588
5.5	0.077	0.088	0.038	0.526
6.0	0.081	0.122	0.027	0.379
6.5	0.088	-	-	-

Table XXI. Weight fraction nitrogen in biomass from integrated and instantaneous balances and instantaneous biomass energetic based on nitrogen and organic substrate measurement for batch fermentation of Candida utilis on filtered glucose from corn dust III; Run #2.

θ, hr	$\frac{N_o - N}{X_\theta - X_o}$, g/g	$\frac{Q_N}{\mu}$, g/g	$\frac{Q_N}{Q_s}$, g/g	η_{N_2}
1.0	0.087	0.094	0.041	0.577
2.0	0.092	0.093	0.028	0.388
3.0	0.094	0.075	0.037	0.519
4.0	0.078	0.085	0.042	0.587
4.5	0.084	0.094	0.045	0.630
5	0.083	0.104	0.044	0.625
5.5	0.079	0.100	0.043	0.607
6.0	0.089	0.087	0.043	0.596
6.3	0.088	0.095	0.036	0.501
6.5	0.088	-	-	-
7.0	0.073	-	-	-
8.0	0.063	-	-	-

Table XXII. Weight fraction nitrogen in biomass from integrated and instantaneous balances and instantaneous biomass energetic yield based on nitrogen and organic substrate measurements for batch fermentation of Candida utilis on filtered glucose from corn dust III; Run # 3.

Θ, hr	$\frac{N_0-N}{X-X_0}$, $\frac{g}{g}$	$\frac{Q_N}{\mu}$, $\frac{g}{g}$	$\frac{Q_N}{Q_S}$, $\frac{g}{g}$	η_{N_2}
1.0	0.086	0.102	0.042	0.592
2.0	0.091	0.100	0.047	0.657
3.0	0.089	0.087	0.040	0.561
4.0	0.094	0.111	0.045	0.630
5.0	0.094	0.098	0.042	0.591
5.5	0.093	0.097	0.050	0.692
6.0	0.095	0.122	-	-
6.5	0.107	-	-	-
7.5	0.106	-	-	-
8.0	0.109	-	-	-

Table XXIII. Weight fraction nitrogen in biomass from integrated and instantaneous balances and instantaneous biomass energetic yield based on nitrogen and organic substrate measurements for batch fermentation of Candida utilis on unfiltered glucose from corn dust III; Run #4.

θ,hr	$\frac{N_o - N}{X - X_o}$, $\frac{g}{g}$	$\frac{Q_N}{\mu}$, $\frac{g}{g}$	$\frac{Q_N}{Q_s}$, $\frac{g}{g}$	η_{N_2}
1.0	0.112	0.125	0.049	0.682
2.0	0.092	0.082	0.038	0.533
3.0	0.079	0.076	0.041	0.578
4.0	0.074	0.079	0.041	0.571
5.0	0.070	0.070	0.035	0.493
5.5	0.071	0.076	0.040	0.599
6.0	0.073	0.093	0.048	0.669
6.5	0.080	0.088	0.043	0.605
7.0	0.077	0.094	0.041	0.579
7.5	0.079	-	-	-

Table XXIV. Weight fraction nitrogen in biomass from integrated and instantaneous balances and instantaneous biomass energetic yield based on nitrogen and organic substrate measurements for batch fermentation of Candida utilis on unfiltered glucose from corn dust III; Run #5.

t, hr	$\frac{N_o - N}{X - X_o}$, $\frac{g}{g}$	$\frac{Q_N}{\mu}$, $\frac{g}{g}$	$\frac{Q_N}{Q_S}$, $\frac{g}{g}$	η_{N_2}
1.0	0.083	0.099	0.022	0.307
2.0	0.088	0.088	0.026	0.363
3.0	0.043	0.070	0.035	0.489
4.0	0.042	0.063	0.031	0.433
5.0	0.060	0.064	0.030	0.419
5.5	0.064	0.071	0.028	0.391
6.0	0.055	0.067	0.031	0.433
6.5	0.056	0.065	0.028	0.391
7.0	0.064	-	-	-
7.5	0.065	-	-	-
8.0	0.062	-	-	-

Table XXV. Weight fraction nitrogen in biomass from integrated and instantaneous balances and instantaneous biomass energetic yield based on nitrogen and organic substrate measurements for batch fermentation of Candida utilis on unfiltered glucose from corn dust III; Run #6.

θ, hr	$\frac{N_o - N}{X - X_o}$, $\frac{g}{g}$	$\frac{Q_N}{\mu}$, $\frac{g}{g}$	$\frac{Q_N}{Q_S}$, $\frac{g}{g}$	η_{N_2}
1	0.089	0.094	0.044	0.62
2	0.087	0.082	0.040	0.56
3	0.088	0.090	0.034	0.48
4	0.112	0.080	0.029	0.41
5	0.083	0.062	0.028	0.39
5.5	0.071	0.055	0.028	0.39
6	0.064	0.044	0.057	0.37
6.5	0.068	-	-	-
7	0.067	-	-	-
8	0.062	-	-	-
9	0.066	-	-	-

Table XXVI. Integrated nitrogen balances in batch fermentations of C. utilis on unfiltered glucose from corn dust III.

Run #	Total nitrogen used (g/ℓ) based on % nitrogen in products.	Total nitrogen used (g/ℓ) based on nitrogen measured in the medium.
4	0.426	0.392
5	0.413	0.379
6	0.435	0.366

Table XXVII. Average weight fraction nitrogen in biomass and biomass energetic yield for Candida utilis growth on filtered and unfiltered glucose from corn dust III.

Run	Based on product nitrogen analysis		Based on integrated consumption of soluble nitrogen		Based on average of instantaneous values	
	N	η	N	η	N	η
1	--	--	0.083	0.58	0.085	0.56
2	--	--	0.083	0.59	0.092	0.56
3	--	--	0.087	0.60	0.102	0.62
4	0.088	0.62	0.081	0.57	0.087	0.59
5	0.068	0.42	0.062	0.38	0.084	0.40
6	0.093	0.61	0.078	0.51	0.072	0.46

Table XXVIII. Values of True Biomass energetic Yield Coefficient (η_{max}) and Maintenance Coefficient, (m_e) from growth of Candida utilis on corn dust III.

Source of data	point estimates η_{max}	e	confidence intervals		r^2	Remarks (data)
Filtered Glucose (Runs 1-3)	0.598	0.036	$0.51 \leq \eta_{max} \leq 0.71$	$0 \leq m_e \leq 0.143$	0.890	Substrate, biomass
	0.589	0.026	$0.54 \leq \eta_{max} \leq 0.65$	$0 \leq m_e \leq 0.088$	0.961	O_2, biomass
	0.640	0.034	$0.59 \leq \eta_{max} \leq 0.70$	$0 \leq m_e \leq 0.085$	0.969	CO_2, biomass
	0.699	0.167	$0.56 \leq \eta_{max} \leq 0.88$	$0.019 \leq m_e \leq 0.32$	0.796	Nitrogen-substrate-biomass
Filtered Glucose (Runs 1-3) -outliers deleted	0.626	0.051	$0.56 \leq \eta_{max} \leq 0.71$	$0 \leq m_e \leq 0.128$	0.949	Substrate, biomass
	0.575	0.006	$0.53 \leq \eta_{max} \leq 0.63$	$0 \leq m_e \leq 0.069$	0.965	O_2, biomass
	0.636	0.031	$0.59 \leq \eta_{max} \leq 0.69$	$0 \leq m_e \leq 0.082$	0.974	CO_2, biomass
	0.652	0.121	$0.53 \leq \eta_{max} \leq 0.84$	$0 \leq m_e \leq 0.259$	0.867	N_2-Substrate-biomass
	0.619	0.043	$0.58 \leq \eta_{max} \leq 0.66$	$0 \leq m_e \leq 0.076$	---	combined estimates(b)
Unfiltered Glucose Runs (4-6)	0.545	-0.050	$0.45 \leq \eta_{max} \leq 0.68$	$0 \leq m_e \leq 0.132$	0.900	Substrate, biomass
	0.789	0.201	$0.68 \leq \eta_{max} \leq 0.88$	$0.103 \leq m_e \leq 0.299$	0.937	O_2, biomass
	0.855	0.166	$0.74 \leq \eta_{max} \leq 0.88$	$0 \leq m_e \leq 0.260$	0.932	CO_2, biomass
	0.379	-0.253	$0.29 \leq \eta_{max} \leq 0.50$	$0 \leq m_e \leq 0.095$	0.844	N_2-Substrate-biomass
	0.831	0.158	$0.73 \leq \eta_{max} \leq 0.88$	$0 \leq m_e \leq 0.237$	0.956	CO_2, biomass (a)
	0.741	0.142	(c)	$0.065 \leq m_e \leq 0.164$	--	Combined estimates (b)

(a) outliers deleted; (b) These are 90% confidence intervals which were obtained from the 97.5% confidence intervals shown above. (c) confidence interval could not be determined.

Table XXIX. Product yield and protein content for the growth of Candida utilis on unfiltered glucose from hydrolysis of corn dust III.

Run	% Protein in dry dust	% Protein in Protein rich Final Product		% Protein in solid at the start of fermentation		g Protein rich Product g dry grain dust		g Protein in Product g dry grain dust		g Protein Produced g dust expended
		Predicted Value	Experimental Value	Predicted	Experimental	Predicted Value	Experimental Value	Predicted Value	Experimental value	
4	6.54	33.04	41.569	18.411	27.1125	0.728	0.591	0.241	0.246	0.444
5	6.54	35.08	38.3813	18.411	29.169	0.699	0.553	0.245	0.212	0.329
6	6.50	34.97	39.175	18.411	28.350	0.700	0.593	0.245	0.233	0.414

Run	Available electrons of protein produced per available electron of dust expended.
4	0.574
5	0.426
6	0.536

Table XXX. Data used for parameter estimation in batch fermentation of Candida utilis on filtered glucose.

μ	Q_s	Q_{O_2}	Q_{CO_2}	η_{N_2}	$\eta + \varepsilon$	$y_c + d$
0.3571	0.580*	9.10	7.40*	0.711*	1.21	1.09
0.3312	0.611	8.57	7.48	0.571	1.06	0.99
0.3013	0.602	8.56	8.02	0.520	1.03	0.97
0.303	0.606	9.07	8.47	0.514	1.02	0.99
0.280	0.592	9.32	8.56	0.588	1.03	0.97
0.274	0.644	9.68	8.89	0.526	0.96	0.910
0.730	1.48	19.36	19.14	0.519	0.98	0.95
0.60	1.18	15.24	12.12	0.587	1.00	0.93
0.54	1.12	18.40	15.41	0.630	1.07	0.96
0.42	0.98	17.11	14.26	0.625	1.04	0.93
0.49	0.99	17.76	15.67	0.607	1.00	0.96
0.48	0.88	15.11	13.44	0.596	1.04	0.97
0.43	1.14*	13.87	12.31	0.501*	0.82	0.76
0.45	1.12*	13.93	12.80	-	0.85	0.81
0.18	0.44	9.20*	6.88	-	1.12	0.94
0.17	0.41	4.59	5.10	0.287*	0.81	0.86
0.24	0.52*	6.45	6.12	0.365	0.94	0.90
0.301	0.65	7.49	6.69	0.537	0.90	0.84
0.304	0.75	8.82	8.32	0.483	0.84	0.86
0.32	0.73	8.15	8.37	0.542	0.85	0.84
0.29	0.57	8.01	8.52	0.419	0.93	0.94
0.25	0.41	8.36	8.90*	-	1.35	1.36

* Outliers deleted during regression analysis.

Table XXXI. Data used for parameter extimation of batch fermentation of Candida utilis on unfiltered glucose.

μ	Q_s	Q_{O_2}	Q_{CO_2}	η_{N_2}	$\eta + \varepsilon$	$y_c + d$
0.74	1.60	15.43	11.94	0.533	0.85	0.76
0.57	1.05	11.63	7.16*	0.578	0.98	0.84
0.40	0.77	13.21	8.96	0.571	1.14	0.95
0.35	0.69	12.13	8.67	0.493	1.10	0.95
0.33	0.59	11.95	9.61	0.559	1.20	1.05
0.33	0.63	11.45	9.57	0.669	1.16	1.06
0.30	0.60	11.40	8.94	0.605	1.16	1.00
0.26	0.59	10.70	9.53	0.578	1.07	0.99
0.56	1.40	18.29	15.75	0.419	1.00	0.88
0.61	1.34	17.87	13.78	0.391	0.87	0.80
0.41	0.90	11.94	10.58	0.433	0.94	0.88
0.37	0.86	11.18	10.16	0.391	0.91	0.86
0.71	1.57	16.56	14.22	0.390	0.86	0.80
0.60	1.19	11.98	10.45	0.390	0.90	0.84
0.56	0.92	10.34	9.27	0.370	1.07	1.00

*Outliers deleted during regression analysis.

APPENDIX

For the estimation of the growth parameters η_{max}, the true biomass energetic yield, and m_e, the energetic maintenance coefficient, from the experimental data, equations (20 - 27) can be employed. These equations can be put in the form

$$\frac{\mu}{\eta_{N_2}} = m_e + \frac{\mu}{\eta_{max}} + \varepsilon_1 \tag{33}$$

$$\frac{\sigma_s \gamma_s}{\sigma_b \gamma_b} Q_s = m_e + \frac{\mu}{\eta_{max}} + \varepsilon_2 \tag{34}$$

$$\frac{48\, Q_{O_2}}{\sigma_b \gamma_b} + \mu = m_e + \frac{\mu}{\eta_{max}} + \varepsilon_3 \tag{35}$$

$$\frac{12\, \gamma_s}{\sigma_b \gamma_b} Q_{CO_2} + \frac{\gamma_s}{\gamma_b} \mu = m_e + \frac{\mu}{\eta_{max}} + \varepsilon_4 \tag{36}$$

where ε_1, ε_2, ε_3, and ε_4 are correlated error terms with mean $\underline{0}$ and variance covariance matrix $\underline{\Sigma}$. The above relationships can be written in terms of a multivariate linear model (25)

$$\underline{Y} = \underline{X}\,\underline{B} + \underline{E} \tag{37}$$

where $\underline{Y}$ is n x 4 response matrix which may contain n observations of

$$\left[\frac{\mu}{\eta_{N_2}},\ \left(\frac{\sigma_s \gamma_s}{\sigma_b \gamma_b} Q_s\right),\ \left(\frac{48\, Q_{O_2}}{\sigma_b \gamma_b} + \mu\right),\ \left(\frac{12\, \gamma_s}{\sigma_b \gamma_b} Q_{CO_2} + \frac{\gamma_s \mu}{\gamma_b}\right)\right]$$

, $\underline{X}$ is an n x 2 matrix with ones in the first column and the values of μ in the second, i.e.,

$$\underline{X} = \begin{Bmatrix} 1 & \mu_1 \\ 1 & \mu_2 \\ \cdot & \cdot \\ \cdot & \cdot \\ \cdot & \cdot \\ 1 & \mu_n \end{Bmatrix}, \tag{38}$$

$$\underline{B} = \begin{Bmatrix} m_e & m_e & m_e & m_e \\ \frac{1}{\eta_{max}} & \frac{1}{\eta_{max}} & \frac{1}{\eta_{max}} & \frac{1}{\eta_{max}} \end{Bmatrix} \tag{39}$$

is a (2 X 4) matrix of unknown parameters, and

$$\underline{E} = \begin{Bmatrix} \underline{\varepsilon}_1' \\ \underline{\varepsilon}_2' \\ \cdot \\ \cdot \\ \underline{\varepsilon}_n' \end{Bmatrix} \tag{40}$$

where $\underline{\varepsilon}_i$ have identical independent normal distributions with mean of $\underline{0}$ and variance-covariance matrix $\underline{\Sigma}$. Estimates of $\underline{B}$ and $\underline{\Sigma}$ can be obtained using multivariate regression techniques (26), and are given by

$$\hat{\underline{B}} = \begin{Bmatrix} \hat{m}_e & \hat{m}_e & \hat{m}_e & \hat{m}_e \\ \frac{1}{\hat{\eta}_{max}} & \frac{1}{\hat{\eta}_{max}} & \frac{1}{\hat{\eta}_{max}} & \frac{1}{\hat{\eta}_{max}} \end{Bmatrix} \tag{41}$$

and $$\hat{\underline{\Sigma}} = \underline{Y}' \left[I - \underline{X}(\underline{X}'\underline{X})^{-1}\underline{X}' \right] \underline{Y}/(n-2). \tag{42}$$

Four different unbiased estimators of $\frac{1}{\eta_{max}}$ and m_e are obtained using the elements of $\hat{\underline{B}}$. These estimators are correlated and can be combined as follows to obtain a single "best" estimator of $\frac{1}{\eta_{max}}$ and m_e. For example if the four different estimators of $\frac{1}{\eta_{max}}$ are denoted by $\hat{\alpha}_1$, $\hat{\alpha}_2$, $\hat{\alpha}_3$, and $\hat{\alpha}_4$, then a linear model of the form

$$\hat{\underline{\alpha}} = \underline{1} \cdot \frac{1}{\eta_{max}} + \underline{\varepsilon} \tag{43}$$

where $\hat{\underline{\alpha}} = [\hat{\alpha}_1 \ \hat{\alpha}_2 \ \hat{\alpha}_3 \ \hat{\alpha}_4]$, $\underline{1}$ is a (4 X 1) column vector of ones, and $\underline{\varepsilon}$ is an error vector with mean $\underline{0}$ and variance-covariance matrix C $\underline{\Sigma}$ (where C is a known constant) can be employed to obtain the overall estimate.

Using the method of generalized least squares, the estimated best linear unbiased estimate (BLUE) of $\frac{1}{\eta_{max}}$ based on $\hat{\alpha}_1$, $\hat{\alpha}_2$, $\hat{\alpha}_3$, and $\hat{\alpha}_4$ is obtained to be (23)

$$\frac{\hat{1}}{\eta_{max}}_k = (\underline{1}' \hat{\underline{\Sigma}}_k^{-1} \underline{1})\, \underline{1}'\, \hat{\underline{\Sigma}}_k^{-1}\, \hat{\underline{\alpha}} \qquad (44)$$

where $\hat{\underline{\Sigma}}_k = \hat{\underline{\Sigma}} + k \text{ diag. } (\hat{\underline{\Sigma}}) \qquad k = [0, \infty]$ (45)

is an estimate of the variance-covariance matrix. A value of $k = \infty$ completely supresses the offdiagonal elements of the matrix and a value of $k = 0$ yields the MLE of $\frac{1}{\eta_{max}}$.

To obtain a combined confidence interval for $\frac{1}{\eta_{max}}$, the 97.5% confidence intervals are obtained using the four different estimates. If the intervals are written as follows (23):

$$A < \frac{1}{\eta_{max}} < B \text{ from substrate nitrogen-biomass data}$$

$$C < \frac{1}{\eta_{max}} < D \text{ from substrate-biomass data}$$

$$E < \frac{1}{\eta_{max}} < F \text{ from } O_2\text{-biomass data}$$

and

$$G < \frac{1}{\eta_{max}} < H \text{ from } CO_2\text{-biomass data}$$

Then,

$$P[A < \frac{1}{\eta_{max}} < B,\ C < \frac{1}{\eta_{max}} < D,\ E < \frac{1}{\eta_{max}} < F, \text{ and } G < \frac{1}{\eta_{max}} < H] \geq 0.90.$$

That is,

$$P[\text{Max } (A,C,E,G) \leq \frac{1}{\eta_{max}} \leq \text{Min}(B,D,F,H)] \geq 0.90$$

and as such the 90% confidence interval can be constructed.

Similarly if the four different estimates of m_e are denoted by $\hat{B}_1$, $\hat{B}_2$, $\hat{B}_3$, and $\hat{B}_4$, the linear model

$$\underline{\hat{B}} = \underline{1}\, m_e + \underline{\varepsilon} \tag{46}$$

where $\underline{\hat{B}}' = (\hat{B}_1, \hat{B}_2, \hat{B}_3, \hat{B}_4)$ and other vectors as defined above can be used to obtain an overall estimator of m_e. The estimated best linear unbiased estimate (BLUE) of m_e is

$$\hat{m}_{e_k} = (\underline{1}\, \hat{\underline{\underline{\Sigma}}}_k^{-1}\, \underline{1})^{-1}\, \underline{1}'\, \hat{\underline{\underline{\Sigma}}}_k^{-1}\, \underline{\hat{B}} \tag{47}$$

Also a confidence interval for m_e can be obtained as done for $\frac{1}{\eta_{max}}$ above.

In the analysis of data, outliers were identified and deleted. This was done by examining a computer plot of the data and deleting any observations that both showed great deviation from the rest of the data and failed to satisfy the consistency tests. The requirement that the sums $(y_c + d)$ and $(\eta + \varepsilon)$ be close to one when no products are formed was combined with the level of deviation from other observations in the decision making process of retaining or deleting an observation during parameter estimation.

The methodology outlined above was used for estimating the true biomass energetic yield (η_{max}) and the energetic maintenance coefficient (m_e). This technique was used also to combine the experimental values of η_{N_2}, Q_s, Q_{O_2}, and Q_{CO_2} into one estimate of η_{max} and m_e. Confidence intervals were constructed for the estimates using the technique outlined.

MICROBIAL CONVERSION OF GRAIN DUST TO USEFUL PRODUCTS

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Literature on the yield of microbial biomass on glucose and other carbohydrates was reviewed. The biomass energetic yield, η, was calculated from other reported yields. For aerobic cultivation of microogranisms with NH_4^+ as the source of nitrogen, the average biomass energetic yield was 0.587 while for anaerobic growth it was 0.184. The aerobic growth yields supported the idea that P/O ratio for microbial growth is around 2.0. It was also found that the true biomass energetic yield η_{max} and maintenance coefficient m_e depend on experimental conditions. Several ways to check the consistency of experimental data using the concept of available electron and the associated regularities were presented.

The microbial conversion of grain dust to useful products such as protein feed supplements and ethyl alcohol is an important grain dust utilization alternative. In this work microbial utilization of corn, wheat and milo dusts was investigated. Enzymatic hydrolysis and microbial growth on the hydrolyzed starch were carried out and yield values were measured and compared to literature values and maximum theoretical values. Growth yields on the filtrate from starch hydrolysis are similar to literature values for carbohydrate substrates such as glucose. Shake flask growth experiments without filtration on the products of starch hydrolysis resulted in protein enriched products with 19 to 31% protein. The best results were obtained with corn dust.

Candida utilis was cultivated in an instrumented fermentor on both filtered and unfiltered substrate obtained from enzymatic hydrolysis of starch in corn dust. For growth on filtered substrate, the average integrated biomass energetic value based on biomass-substrate data was $\eta = 0.55$ and for growth on unfiltered substrate an average yield value of $\eta = 0.59$ was obtained. Material and energy balances showed that the presence of unfiltered corn residue in the media had no significant effect on the yields. Statistical methods were developed and used to obtain best estimates of the growth parameters.

Values of the biomass energetic yield corrected for maintenance, η_{max} = 0.619, and the maintenance coefficient, m_e = 0.043, were obtained for growth on filtered substrate. The consistency of data and parameter estimates were relatively good for filtered substrate, however, the parameter estimates for unfiltered substrate were not consistent. Growth experiments without filtration of the products of starch hydrolysis resulted in protein enriched products with about 39.73% protein.