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Carbon Dioxide Evolution Rate As a Method to Monitor and Control an Aerobic Biological Waste Treatement System

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INTRODUCTION

A long term manned space mission(ca.2 to 20 years) may require a regenerative life support system(RLSS). A closed ecological life support system(CELSS) or partially closed ecological life support system(PCELSS) may be suitable for this purpose. In a partial CELSS a significant portion of the food is expected to be grown on board. Waste material, principally consisting of inedible plant residues and human metabolic wastes, must be treated to yield CO_2 plus a mineral solution capable of supporting plant growth if acceptable system closure is to be attained.

Shuler(1979) has reviewed the advantages and disadvantages of physical- chemical and biological waste treatment systems within the context of CELSS. He suggested that generally a wet-oxidation process (physical- chemical) approach might be anticipated to be more space efficient but the form of outputs from biological processing may be more suitable to support plant growth (probably hydroponics).

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The objective of this investigation is to develop a mathematical model that can predict the performance of the biological waste treatment reactor which handles the complex chemically undefined feed. The reactor uses an undefined mixed culture of microorganisms. In this paper we will discuss how a simple carbon mass balance and carbon dioxide evolution information can be used as tools in modelling such a complex mixed culture and mixed substrate bioprocess. We will also present a mathematical model and compare the model predictions to experimental results both for steady-state and for transient conditions.

MODELLING APPROACH

Due to the complexity of the waste material to be biologically regenerated, the model is semi-empirical. Fig.1 illustrates our overall approach to modelling this system. The feed is considered to be composed of three major components: soluble and insolubles either non-lignocellulosics or lignocellulosics. Insoluble substrates must be hydrolyzed to soluble form before they can be utilized since we assume that the microbes will utilize only the soluble form of substrate. Therefore

OVERALL MODELLING APPROACH

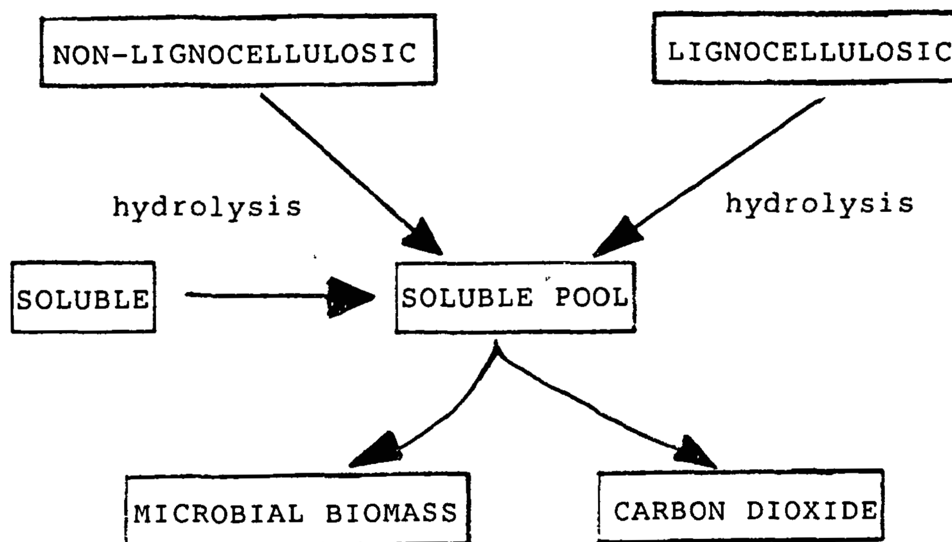


Figure 1. Overall modelling approach

the amount of insoluble non- lignocellulosic and lignocellulosic substrate utilized can be no more than than the amount hydrolyzed. Contributing to the total pool of soluble substrate is the soluble portion of waste in the feed and the hydrolysis products of insoluble non-lignocellulosics and lignocellulosics.

Therefore our model will be developed in two steps. The microbial growth model from only soluble substrate will be developed first, then we will develop the hydrolysis kinetic model of insolubles which will be coupled with the microbial growth model to describe the overall biodegradation of waste material.

CLEAN SYSTEM

To study the microbial growth kinetics from the soluble substrate, we have worked with a "clean system". In the "clean system" experiments we obtained a clarified feed by separating a soluble portion of the "real" waste by filtering the waste through a ultrafiltration unit with 10,000 M.W. cut off membrane. 5g/liter of glucose is added to the soluble portion to simulate the hydrolysis product of cellulose.

There are two major advantages in working with the "clean system". Firstly, it is easier to conduct experiments. The presence of insoluble lignocellulosics in the reactor causes many mechanical problems such as plugging of tubing, difficulties in maintaining homogeneous mixing , etc. Secondly, several different methods of estimating microbial cell mass can be compared. Estimating biomass with precision and accuracy is essential in studying microbial growth kinetics. However, it is very difficult to estimate cell biomass in a complex system where insoluble particles such as lignocellulosics are present in high concentration. One of the methods we have used successfully is a carbon balance and CO_2 evolution rate (CER) method. Using the "clean system" allowed us to test the validity of the carbon balance-CER method by comparing it to methods such as dry weight, and optical density. The advantage of the carbon balance-CER method is that since CER can be measured on-line, the cell biomass and growth rate can be obtained with speed and precision.

MATERIAL AND METHODS

MODEL WASTE

A model waste simulating that expected to be generated in a space ship with 20 crew members during a ten year mission has been formulated. The composition of model waste is given in Table 1. Cerophyl(Cerophyl Lab.,Inc., Kansas City, MO.) is dried and ground stems and leaves from young rye plants. It is used to mimic the inedible plant residues. Freeze- dried feces and urine were obtained from USDA. The food preparation wastes, freeze-dried and canned, were prepared by Prof.Karel of MIT to simulate the types of waste that might result from a predominantly vegetarian diet.

ORGANISMS

A mixed culture was originally obtained from soil and the Trumansburgh, NY activated sludge plant. The mixed culture is stored frozen(-20° C) prior to the inoculation of the experimental system.

TOTAL CARBON

Total carbon was analyzed using a total carbon analyzer Beckman Model 915A. Samples of feed, reactor and recycle

Table 1. Waste Model

Component		Quantity
1. Urine		1.76 g
2. Feces		1.24 g
3. Cerophyl		11.03 g
4. Food preparation waste[+]		1.10 g
5. Wash water concentrate		0.47 g
	Wt. % of wash water concentrate solids	
(a) Lactic Acid	9.0	
(b) Urea	7.0	
(c) Glucose	1.0	
(d) Soap (Ivory)	30.0	
(e) Purified Cellulose	35.0	
(f) NaCl	9.0	
(g) KCl	9.0	
6. Synthetic spent nutrient		0.72 g
	Wt. % of Synthetic spent Nutrient Solids	
(a) NaCl	65.4	
(b) K_2HPO_4	5.0	
(c) $CaSO_4 \cdot 2H_2O$	6.2	
(d) $MgSO_4 \cdot 7H_2O$	3.8	
(e) $FeSO_4 \cdot 7H_2O$	0.06	
(f) H_3BO_3	0.02	
(g) $ZnCl_2$	0.02	
(h) $CuSO_4 \cdot 5H_2O$	0.01	
(i) Cinnamic Acid	19.5	
7. Distilled-deionized H_2O		1,000 ml

Total Solids - 16.3 g/l; pH = 5.6

[+]Supplied by Prof. Karel at MIT

were hydrolyzed by concentrated H_2SO_4 (98%) immediately after sampling. A 10ml portion of the sample was mixed with 10ml of concentrated H_2SO_4 in a glass vial at room temperature. The vial was shaken gently several times. The acid hydrolyzed samples were stored at room temperature until they were analyzed. A soluble portion was obtained by centrifuging the sample then filtering the supernatant through 0.45 um membrane filter. The soluble sample was then stored frozen(-20° C) prior to analysis.

CO2 MEASUREMENTS

Carbon dioxide concentration in the effluent gas was measured by using a Gas Chromatograph(Aerograph Series 2700, Varian Associates,Inc., Palo Alto, CA), with a thermal conductivity detector (F and M Scientific 700 Laboratory Chromatograph, Hewlett- Packard, Inc.). A Porapak Q column was used.

DISSOLVED OXYGEN (D.O.)

Dissolved oxygen concentration of the reactor was measured with D.O. probe (Leeds and Northrup Electronics, PA) and monitored intermittenly.

OPTICAL DENSITY (O.D.)

Optical density was measured by Bausch and Lomb Spectronic 20 spectrophotometer at 600 nm.

VIABLE CELL COUNT (V.C.C.)

Viable cell count was determined by spread plate method on CGY medium(Unz and Dondero,1967) after mild sonication of the 50ml sample in a 100ml beaker to disperse microbial flocs. A Sonic Dismembrator Model 300 was used with a power setting of 25. The sample beaker was immersed in a water bath at room temperature during the sonication to cool down the sample. The plates were incubated at 30° C for 3 days.

CELLULOSE,HEMICELLULOSE AND LIGNIN

Cellulose, hemicellulose, and lignin were determined by acid- detergent method (Goering and Van Soest,1970). The analysis was performed by Dr.J.Robertson of the department of Animal Science, Cornell University.

CHEMOSTAT EXPERIMENTS

Clean System

Continuous experiments with "clean" feed (soluble portion

of the model waste with 5g/l of glucose added) were conducted using a 2 liter Multi-gen (New Brunswick Sci. Co., New Brunswick, NJ) with a working volume of 1.2 liter. The reactor was controlled for temperature and pH of 7.0 by adding 2N H_2SO_4 as required (via NBS pH controller).

Real System

Continuous experiments with "real" model waste(which includes insoluble fractions) were conducted in a 7.5 liter NBS Model 19 Fermenter operating with 4 liter working volume. The experimental system is shown in Fig 2. pH was maintained at 7.2 by pH controllers (NBS). 2N H2SO4 and 2N NaOH was used for acid and base addition. The temperature was also controlled and dissolved oxygen(D.O.) was monitored intermittenly using a Leeds and Northrup D.O. probe on a slip stream. Aeration was achieved by sparging air directly underneath the bottom impeller. Three impellers (turbine flat blade type) were used for aeration and agitation. Air flow rate was maintained above 1.5 vvm and agitation was higher than or equal to 400 rpm. Air flow rate and agitation were varied to maintain D.O. above 2 mg/liter. Excessive mixing is undesirable since foaming may result.

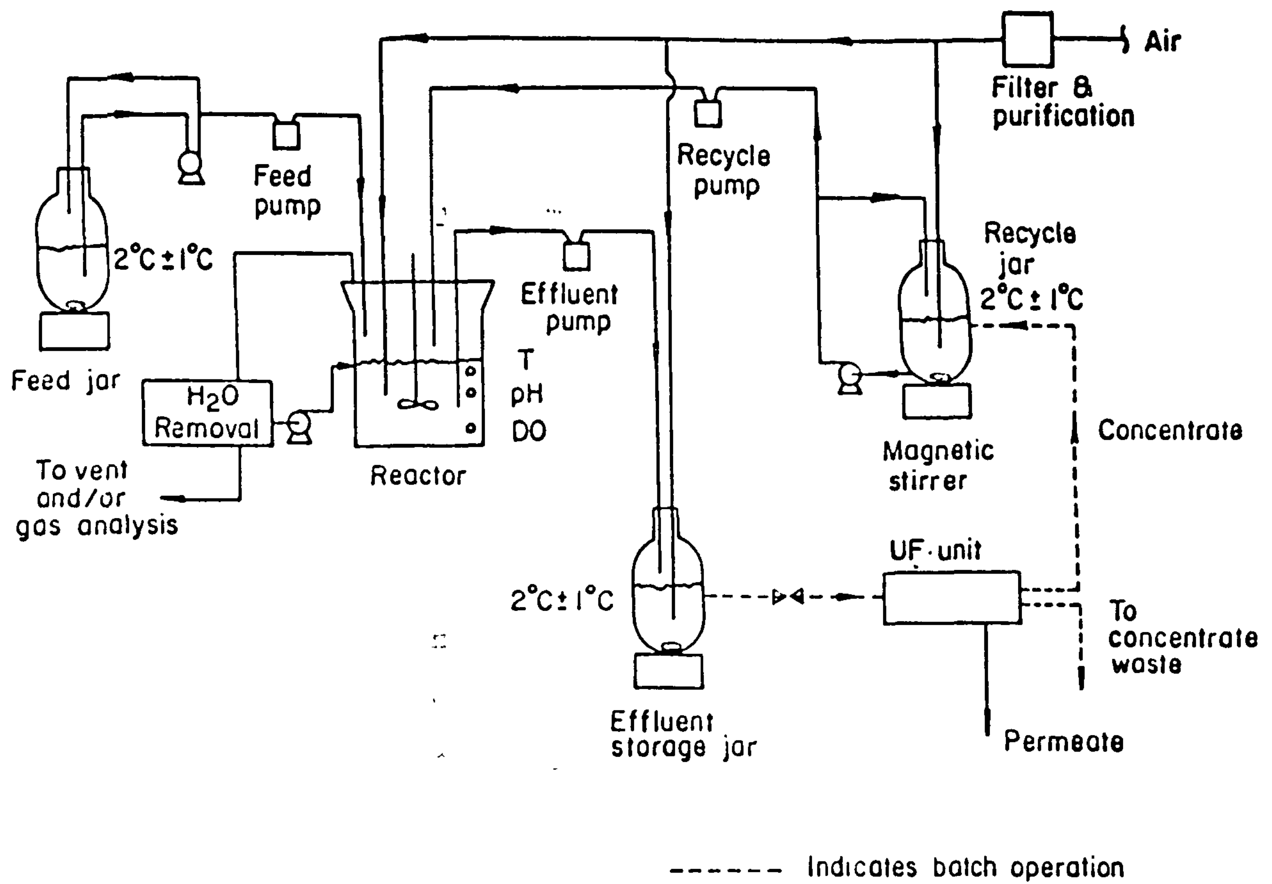


Figure 2. Flow diagram of "real" experimental system

RESULTS AND DISCUSSION

Experimental data from chemostat experiments for the "clean system" are listed on the Table 2. Dilution rate was the independent parameter. Data are from steady-state operation.

TABLE 2: Experimental Results for "Clean System" at 25°C

Θ_H(hr)	S(mg/lℓ)	C.E.R. (mg/ℓ-hr)	Total Carbon in Reactor (mg/ℓ)	Biomass (mg/ℓ)
7.5	400	292	1968	1568
9.7	295	224	1936	1641
11.2	279	191	1636	1357
16.0	407	132	1932	1525
40.8	203	61	1728	1525

Notes:

1. All data expressed as total carbon concentration.
2. Substrate concentration, S, included recalcitrant portion of 200 mg/ℓ.
3. C.E.R. = CO_2 evolution rate.
4. Feed concentration, S_o, was 4200 ppm.
5. Biomass, X, is estimated by simple carbon mass balance (see eq. 1).

CARBON BALANCE

Mass balance of the system is necessary information for a CELSS. Traditional characterization of substrate level as BOD or COD does not provide material balance information. To obtain carbon mass balance, all the samples were analyzed for total carbon. This procedure allows us to estimate the microbial biomass concentration from a simple carbon balance. For the "clean system" :

$$Rc = Xc + Sc \qquad (1)$$

where Rc = carbon content of reactor sample (mg/l)
Xc = microbial biomass as carbon (mg/l)
Sc = soluble substrate concentration as carbon(mg/l)

The microbial biomass, Xc, may be estimated by difference using eq.1, since Rc and Sc can be measured experimentally. Fig.3 shows the microbial biomass estimated by the carbon balance method compared to the experimental results of dry weights(D.W.) measurements and O.D. measurements for the "clean system". The average carbon content of microbial biomass(measured by by D.W.) is calculated to be 38 %. This is lower than the value of 46.2 % reported by Erickson and his co-workers(1979). However, the graph shows that the results of all three different method are consistent with

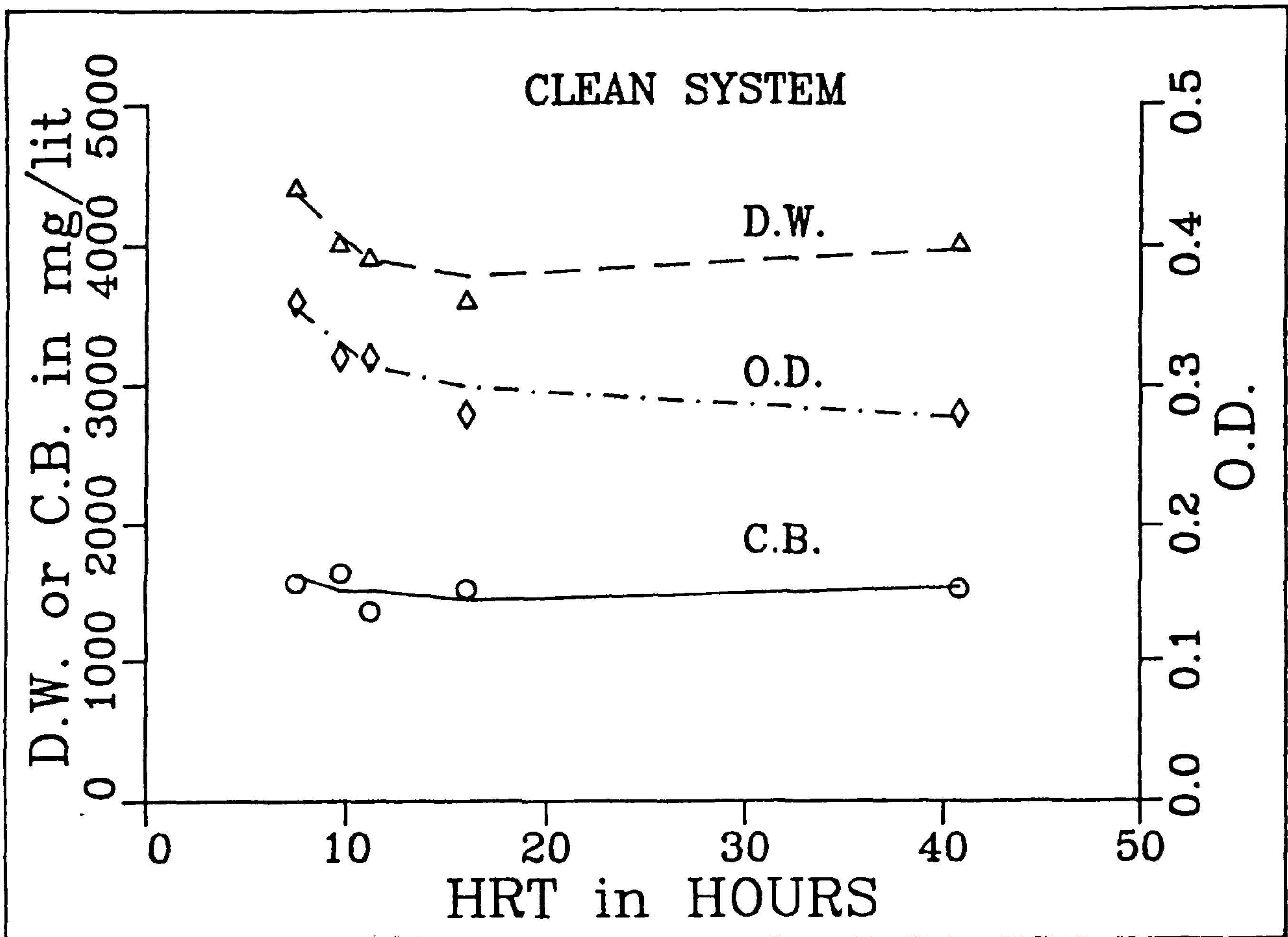


Figure 3. Comparison of different biomass estimation methods: Dry Weight(D.W.), Optical Density(O.D.), and Carbon Balance(C.B.)

each other. The graph also shows that the biomass increases as the dilution rate (D) increases. This may be explained by the assumption that the maintenance energy requirement will be proportionally less as the growth rate increases(Pirt,1965). The carbon balance measurements can be checked for the consistency using carbon dioxide evolution rate(CER) measurements (see eq.5 and Fig.4).

IMPORTANCE OF CER

The carbonaceous substrate may be incorporated into microbial biomass, evolved as respiration by products, or incorporated into extracellular products. The overall balance equation of aerobic microbial reaction may be written as (Erickson et al.,1979)

$$CH_mO_l + aNH_3 + bO_2 = ycCH_pO_nN_g + zCH_rO_sN_t + cH_2O + dCO_2 \quad (2)$$

where CH_mO_l = elemental composition of the organic substrate

$CH_pO_nN_g$ = elemental composition of the cell biomass

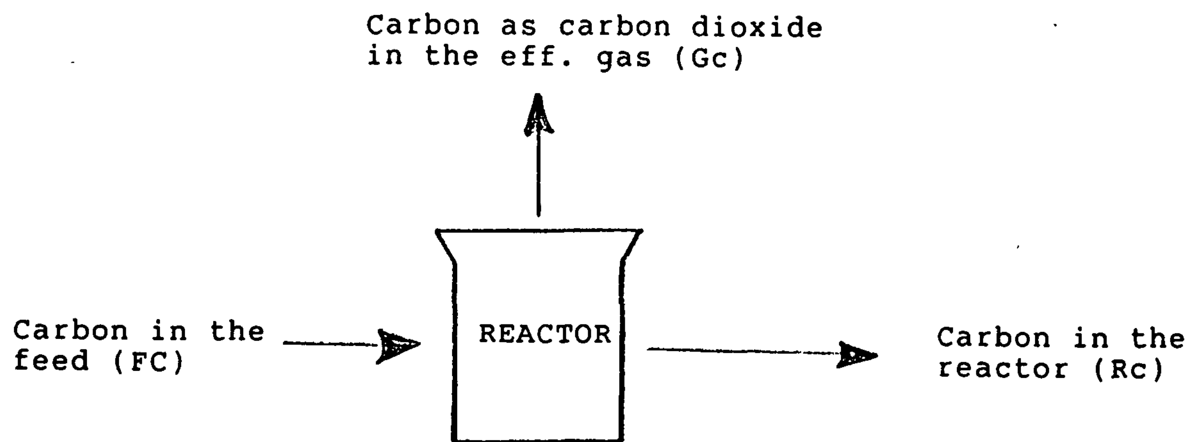
$CH_rO_sN_t$ = elemental composition of any extracellular products

If we consider the carbon balance

$$yc + z + d = 1.0 \quad (3)$$

and when no extracellular products are formed ($z = 0$)

$$yc + d = 1.0 \quad (4)$$



$$Gc = (CER)(HRT) = Fc - Rc$$

Figure 4. Carbon Balance

Eq.4 shows that the amount of carbon in the effluent gas as CO_2 in our "clean system" experiments should be equal to the differential amount of carbon between the feed and the reactor (see Fig.4). The carbon dioxide evolution rate (CER) is obtained by

$$CER(mg/l\text{-}hr) = \text{Carbon in } CO_2/\text{Residence time(HRT)}$$

Therefore CER may be used to check the consistency of the experimental measurements since we may write

$$(CER)(HRT) = Fc - Rc \qquad (5)$$

where Fc = feed concentration as carbon (mg/l)

CER can also be used to estimate the microbial biomass directly once the relationship between the CER and biomass is determined for a given system and a set of environmental conditions. When the substrate acts as both a carbon and energy source for an organism accurate estimation of the part of the carbon source which is assimilated and that part which is dissimilated to provide energy is difficult. However, adequate estimation often can be made with the following method(Pirt,1965).

For an aerobic system we assume that the oxygen is used exclusively as the final electron acceptor in energy yielding process(Hernandez and Johnson,1967) and that the amount of carbon in CO_2 is equivalent to the amount of carbon substrate dissimilated to provide energy. Pirt(1965) has suggested that the substrate used to produce biomass may be composed of two fractions;

total substrate utilized (ΔSt) = substrate utilized to produce cell carbon (ΔSc) + substrate utilized to provide energy (ΔSe) (6)

If we divide the substrate balance by ΔX, that is the amount of biomass produced, we obtain

$$\Delta t / \Delta X = \Delta Sc / \Delta X + \Delta Se / \Delta X \quad (7)$$

which can be written as

$$1/Y = 1/Yc + 1/Ye \quad (8)$$

where Y = overall yield ($\Delta X / \Delta St$)
Yc = biomass yield ($\Delta X / \Delta Sc$)
Ye = energy yield ($\Delta X / \Delta Se$)

for a carbon balance case

$$Yc = 1$$

Then we obtain

$$1/Y = 1 + 1/Ye \quad (9)$$

As discussed earlier

ΔSe = carbon as CO_2 in effluent gas for aerobic system

$$CER = \Delta Se / HRT = (\Delta Se)(D) \quad (10)$$

$$Ye = \Delta X / \Delta Se = (D)(X) / CER \quad (11)$$

Since for chemostat $\Delta X = (X - Xo) = X$, combining eq.11 with eq.9 we obtain

$$1/Y = 1 + CER / (D)(X) \quad (12)$$

It has been postulated (Pirt,1965) that microbes require energy both for growth and for maintenance purposes. Therefore the total substrate dissimilated to provide energy , ΔSe , is assumed to be composed of two fractions: a portion for growth (ΔSeg) and the other portion for maintenance (ΔSem). This can be written as

$$Ye = \Delta X / \Delta Se = \Delta X / (\Delta Seg + \Delta Sem) \quad (13)$$

when the maintenance energy requirement is zero, that is M Sem = 0, we have the 'true' growth yield given by

$$Yeg = \Delta X / \Delta Se \quad (14)$$

combining eq.11,13, and 14 we obtain

$$1/Ye = CER / DX = 1/Yeg + \Delta Sem/X \quad (15)$$

And when we introduce the maintenanace energy coefficient,m(1/ hr), we obtain (Pirt,1965)

$$1/Ye = 1/Yeg + m/\mu = CER/DX \quad (16)$$

and the eq. 12 can be written as

$$1/Y = (1 + 1/Yeg) + m/\mu \qquad (17)$$

since for a chemostat $\mu = D$, we obtain

$$1/Ye = 1/Yeg + m/D = CER/DX \qquad (18)$$

and

$$1/Y = (1 + 1/Yeg) + m/D \qquad (19)$$

The utility of the above two equations is that we can estimate biomass from the CER measurements for a given system, since the overall yield and the yield based on the energy provided (or CO_2 evolved) are expressed as a function of CER and D. When eq.18 and eq.19 are plotted (CER/DX vs 1/D and 1/Y vs 1/D) these two equations should both have the same slopes, m, and their interceptors should have a difference of 1.0 . Fig.5 shows such a plot for the "clean system" at 25° C. This graph demonstrates how CER measurements may be used to check the consistency of the carbon balance experimental results.

We have thus far discussd how CER can be used to check the experimental results of carbon balance method to estimate biomass and how it can be also used to estimate biomass directly once we have obtained Pirt's relationship expressed by eq.18 and eq.19 for a given

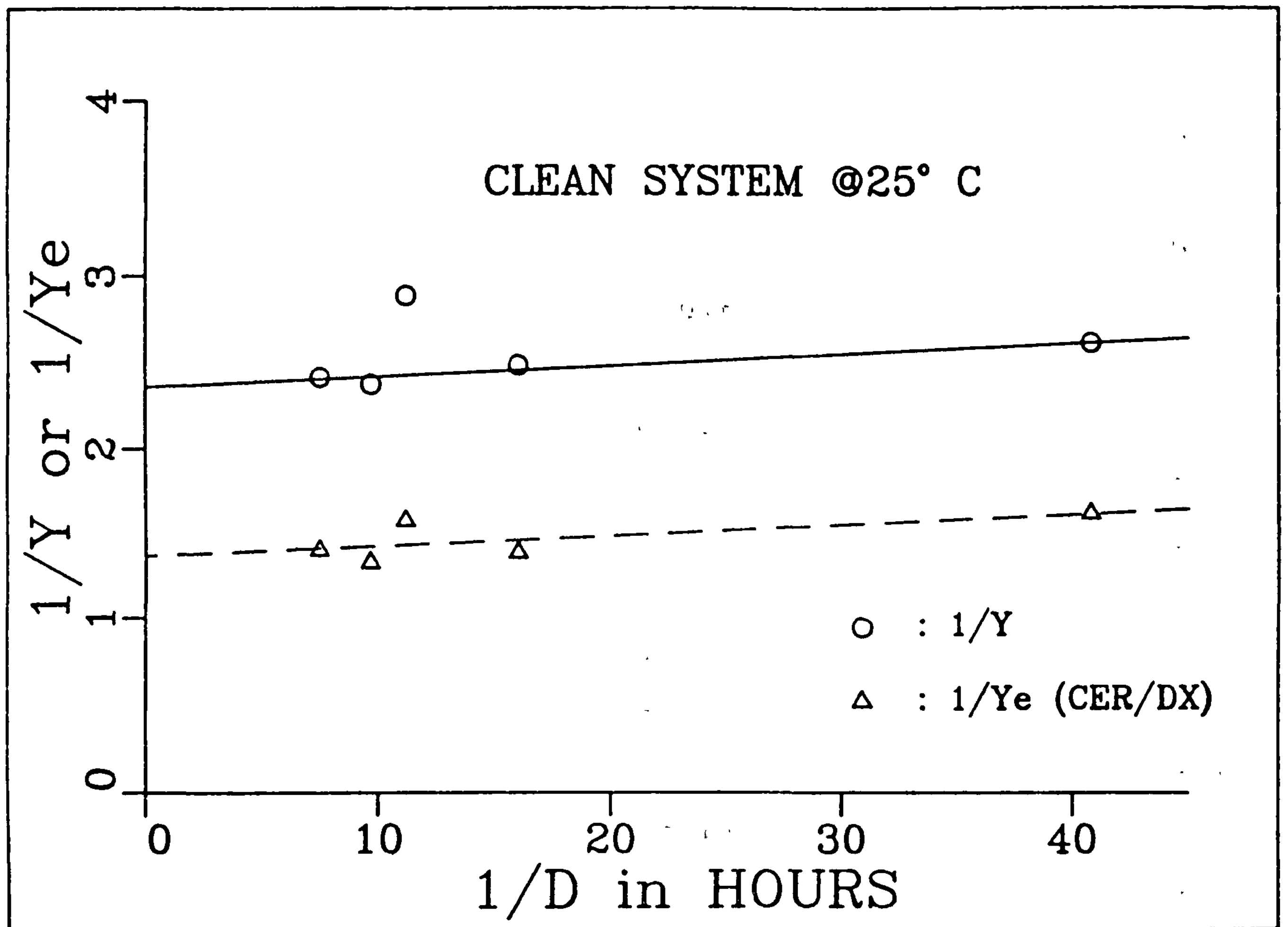


Figure 5. Graph of 1/Y and 1/Ye vs 1/D

experimental system. The advantage of using CER - carbon balance approach is that CER can be measured on-line with speed and precision. Thus biomass, and growth rates may be estimated almost instantaneously.

RELIABILITY OF CER MEASUREMENTS

We have seen that CER can give you a very useful information, and it can be measured on line. It is an excellent candidate for use as a process control parameter. Several investigators have suggested using CER as a process control parameter(Bravard et al., 1979; Mou and Cooney, 1983; Heijnen and Roels, 1979). Some of the criteria for an effective process control parameter is that it must be reliable,reproducible and sensitive to the changes in environmental conditions. Fig.6 shows the experimental results of CER measurements obtained at different hydraulic residence time(HRT) for four different experimental systems. They all display similar response as a function of HRT. The data for the pure culture of E.Coli grown on the glucose limiting media at 37°C is from the work of Domach(1983). CER for this E.Coli system was lower than other systems since the influent substrate concentration, So, was only 1,000 ppm while the So's for the "clean system" were 4,200 ppm.

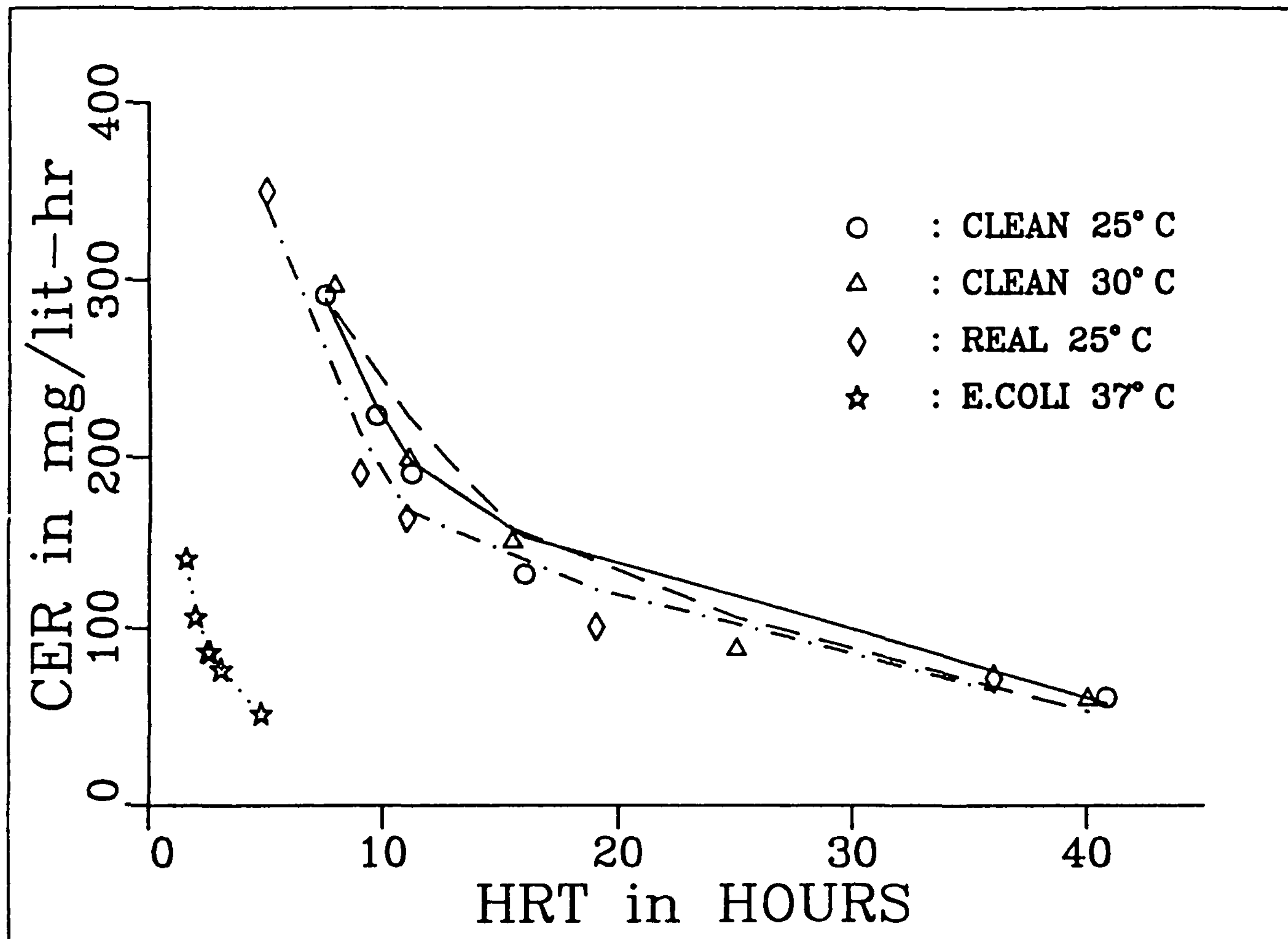


Figure 6. CER measurements vs HRT for four different systems

"So" for the "real system" varied with different HRT's since the amount of lignocellulosic substrate hydrolyzed added to the pool of soluble substrate and varied with HRT. The hydrolysis kinetic model of lignocellulosic substrate to soluble substrate will be presented in a future paper.

MODELLING OF MICROBIAL GROWTH KINETICS

The detailed description and theoretical justification of the model we have used will be discussed in a future paper. For now, we will briefly outline the basic assumptions and mathematical expressions of the model which was originally suggested by Andrews and Tien (1977). However they did not not test the model with chemostat experiments. We have extended the model to predict not only the steady state but also for transient responses, and the experimental verification of the model is discussed in this paper.

We assume that 1) the mixed culture may be represented as a single population of microbes which has the average properties of the mixed culture; 2) the biotic phase is assumed to have two distinctively

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separate compartments; protoplasm which is capable of growth and other metabolic reactions and the stored substrate portion within the cell which is not yet oxidized; and 3) the microbial growth rate is directly proportional to the amount of stored substrate the cell has. The maximum growth rate occurs when the cell has the maximum amount of stored substrate within the cell. Thus:

$$\mu / \mu max = \text{Stored substrate} / \text{Max. stored substrate}$$
$$= (Z)(P) / (Z)max(P) \qquad (20)$$

where μ = specific growth rate (1/hr)
μmax = max. specific growth rate (1/hr)
Z = ratio of stored substrate to the protoplasm concentration
Zmax = max. ratio of stored substrate to the protoplasm concentration

A furthur assumption is that the byproduct of metabolic energy producing reactions is CO_2 and the energy requirement is composed of two portions; growth associated and maintenance associated portions.

The underlying idea of these assumptions is that the microbial growth has two rate limiting reactions. The first one is the uptake rate of soluble substrate into the cell mass. The second one is the growth step where this stored substrate is used to produce additional

protoplasm and CO_2 is generated as a byproduct. Fig.7 illustrates these steps graphically.

The rate expressions for these steps were proposed by Andrews and Tien (1977). The uptake rate of substrate may be written as:

$$Rs = (Ku)(S)(P)(1 - Z/Zmax) \qquad (21)$$

where Rs = substrate uptake rate (mg/l-hr)
Ku = kinetic constant (l/mg-hr)
S = substrate concentration (mg/l)
P = protoplasm concentration (mg/l)

The growth rate may be written as:

$$Rg = (Kg)(Z)(P) \qquad (22)$$

where Rg = growth rate (mg/l-hr)
Kg = kinetic constant (1/hr)

The rate of metabolic reaction by product formation may be written as:

$$CER = (Kr)(Rg)+(Kb)(P) = (Kr)(Kg)(Z)(P)+(Kb)(P) \qquad (23)$$

where Kr = growth associated kinetic constant(mg/mg)
Kb = maintenance associated kinetic constant (1/hr)

Using these rate equations we may write material balance equations for substrate concentration, protoplasm concentration, and storage concentration for chemostat:

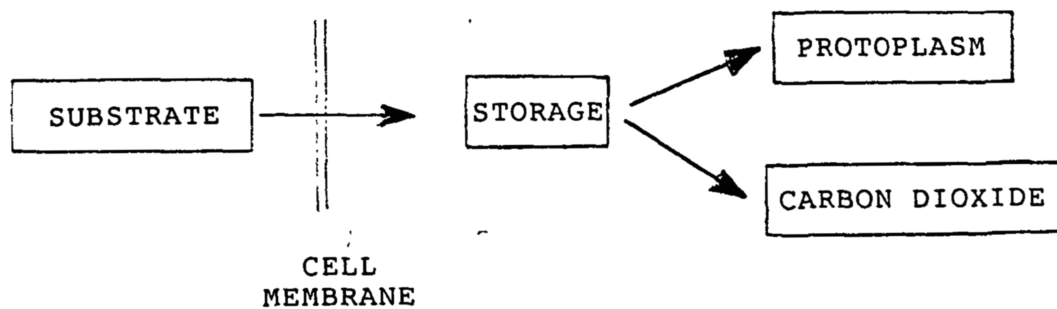

Figure 7. Graphic representation of substrate uptake step and stored substrate utilization step.

$$dS/dt = (D)(So - S) - Rs \quad (24)$$

$$dP/dt = -(D)(P) + Rg \quad (25)$$

$$d(Z)(P)/dt = -(D)(Z)(P) + Rs - Rg - CER \quad (26)$$

Substituting eq.21,22, and 23 into eq.24,25, and 26 we obtain:

$$dS/dt = (D)(So-S) - ((Ku)(S)(P)(1 - Z/Zmax)) \quad (27)$$

$$dP/dt = -(D)(P) - (Kg)(P)(Z) \quad (28)$$

$$dZ/dt = (Ku)(S)(1 - Z/Zmax) - (Z)(Kg) - (Z)(Z)(Kg) - (Kg)(Kr)(Z) - (Kb) \quad (29)$$

To use these equations we need to determine the kinetic parameters Ku,Kg,Kr,and Kb. These can be obtained from steady state chemostat data. Eq.27,28 and 29 can be solved using 4th order Runge-Kutta method.

Fig.8,9 and 10 show the steady-state experimental data compared with the model predictions for the "clean system" at 25° C and 30° C. The overall yield, CER, and 1/D vs 1/S were predicted very well. Biomass is represented as $X = (P)(1 + Z)$ in our model. Perhaps the best way to test the plausibility of a model is to see how well the transient responses can be predicted. Fig.11 shows the experimental CER measurements as a function of time when we increased the dilution rate from 0.0245/hr to 0.1030/hr. It can be seen that CER

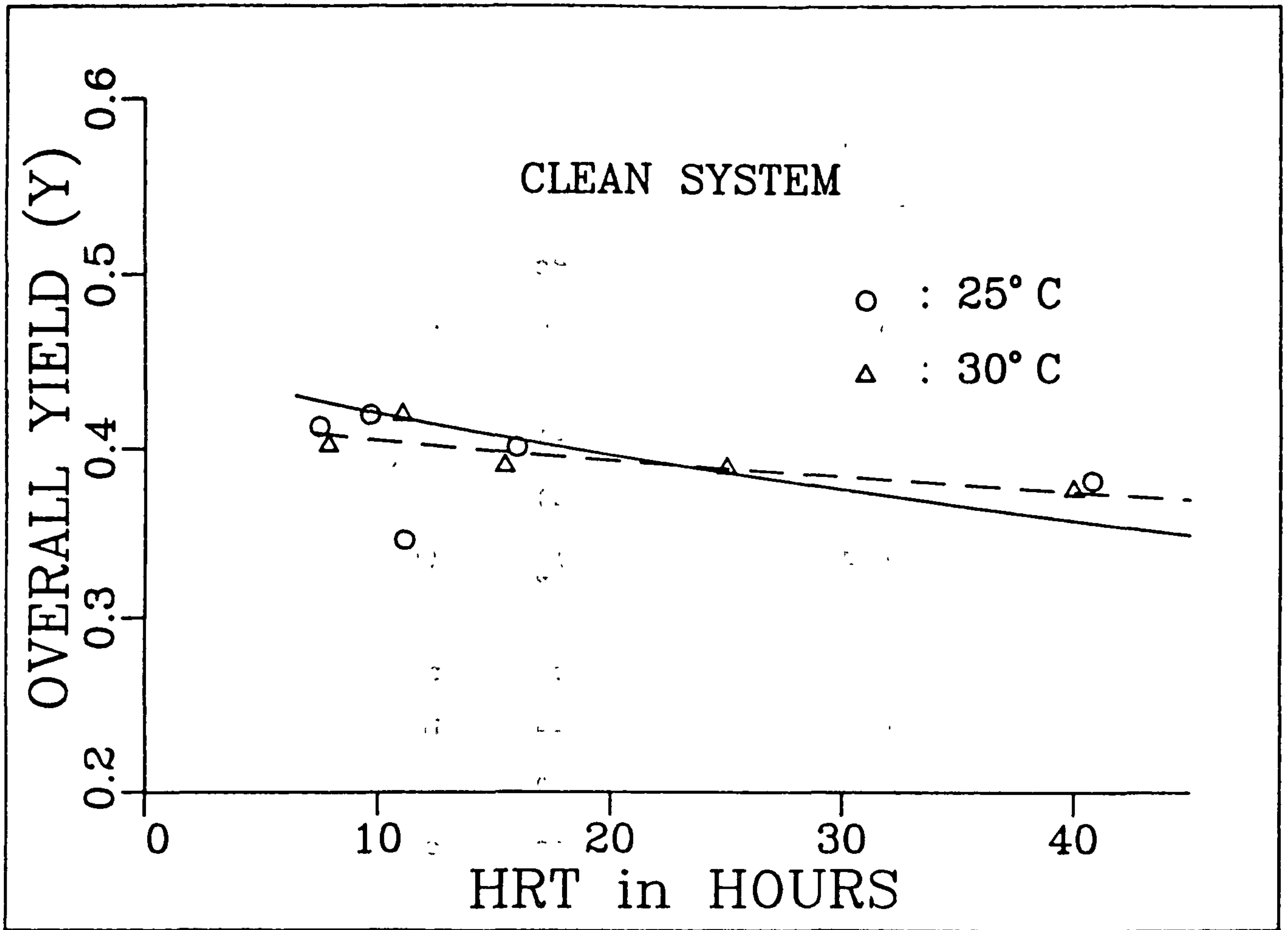

Figure 8. Comparison of experimental data and model prediction for overall yield(Y) for "clean system". Model predictions are represented by solid line for 25° C,and dashed line for 30° C.

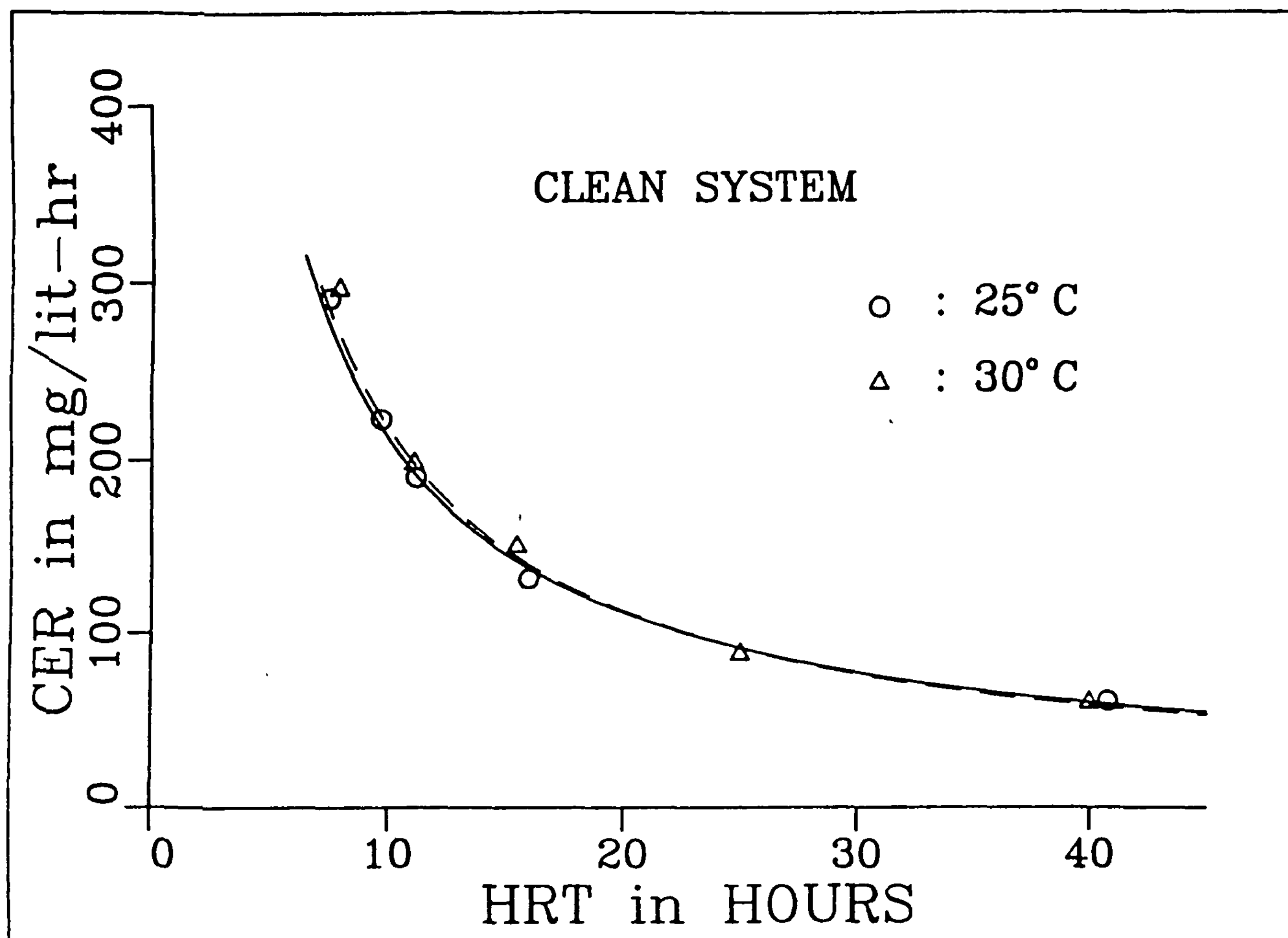

Figure 9. Comparison of experimental data and model predictions for CER for "clean system".Model predictions are represented by solid line for 25° C,and dashed line for 30° C.

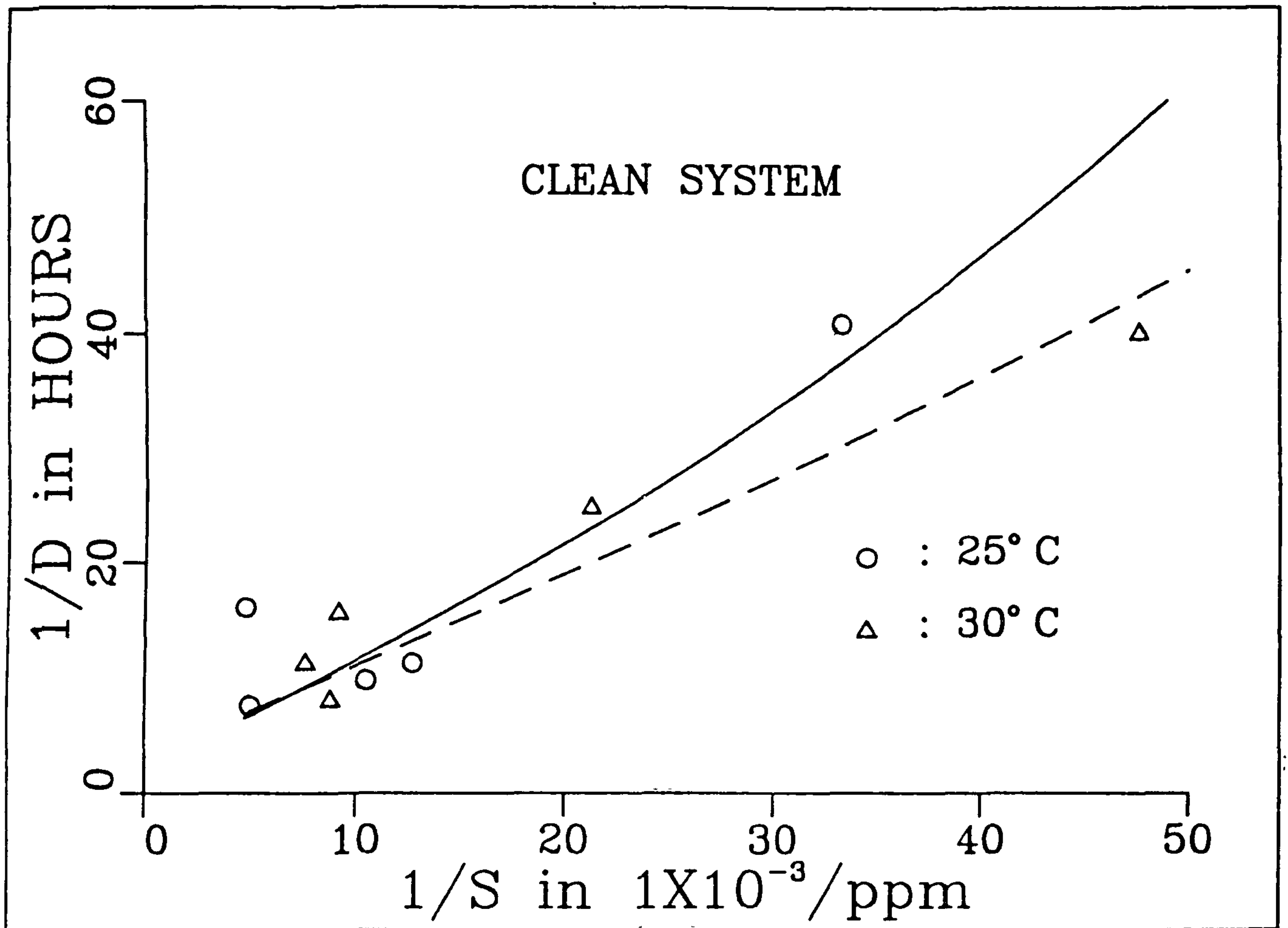

Figure 10. Comparison of experimental data and model predictions for 1/D VS 1/S for "clean system". Model predictions are represented by solid line for 25° C, and dashed for 30° C.

responded almost instantaneously after the increase of the dilution rate. This CER data is reproducible which furthur demonstrates the sensitivity and the reliability of CER measurements. The model predicts the transient response in CER remarkably well. The broken line in Fig.11 is the model prediction using Powell's model(Powell,1969). Powell's model is basically a Monod model modified to account for the time delay in cellular response to changes in growth rates. It is evident that our model predicts the transient response better. Fig.12 shows experimental CER data compared to the model prediction as we decreased the dilution rate from 0.127/hr to 0.025/hr. The model prediction is again excellent. Perhaps the most important results of this investigation is that the model we used can predict the transient response very well without the use of adjustable parameters.

The modelling of "real system" needs to incorporate the hydrolysis kinetic model for lignocellulosic substrate. The hydrolysate production rate equation from the hydrolysis model will be added to eq.27 as an added soluble substrate generation term. The hydrolysis model of lignocellulosic substrate and the overall model have

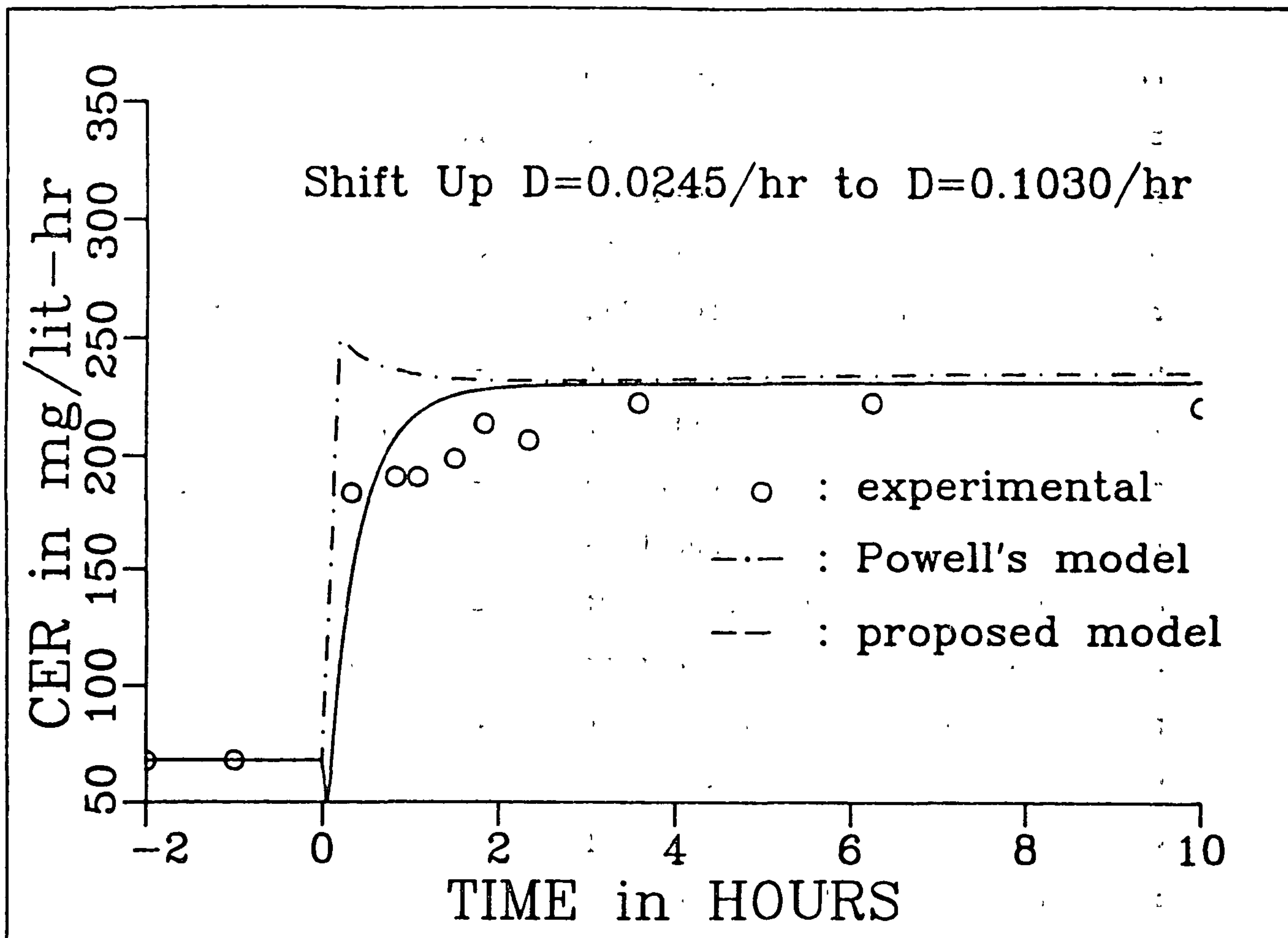

Figure 11. Comparison of CER data and model prediction of shift-up experiments.

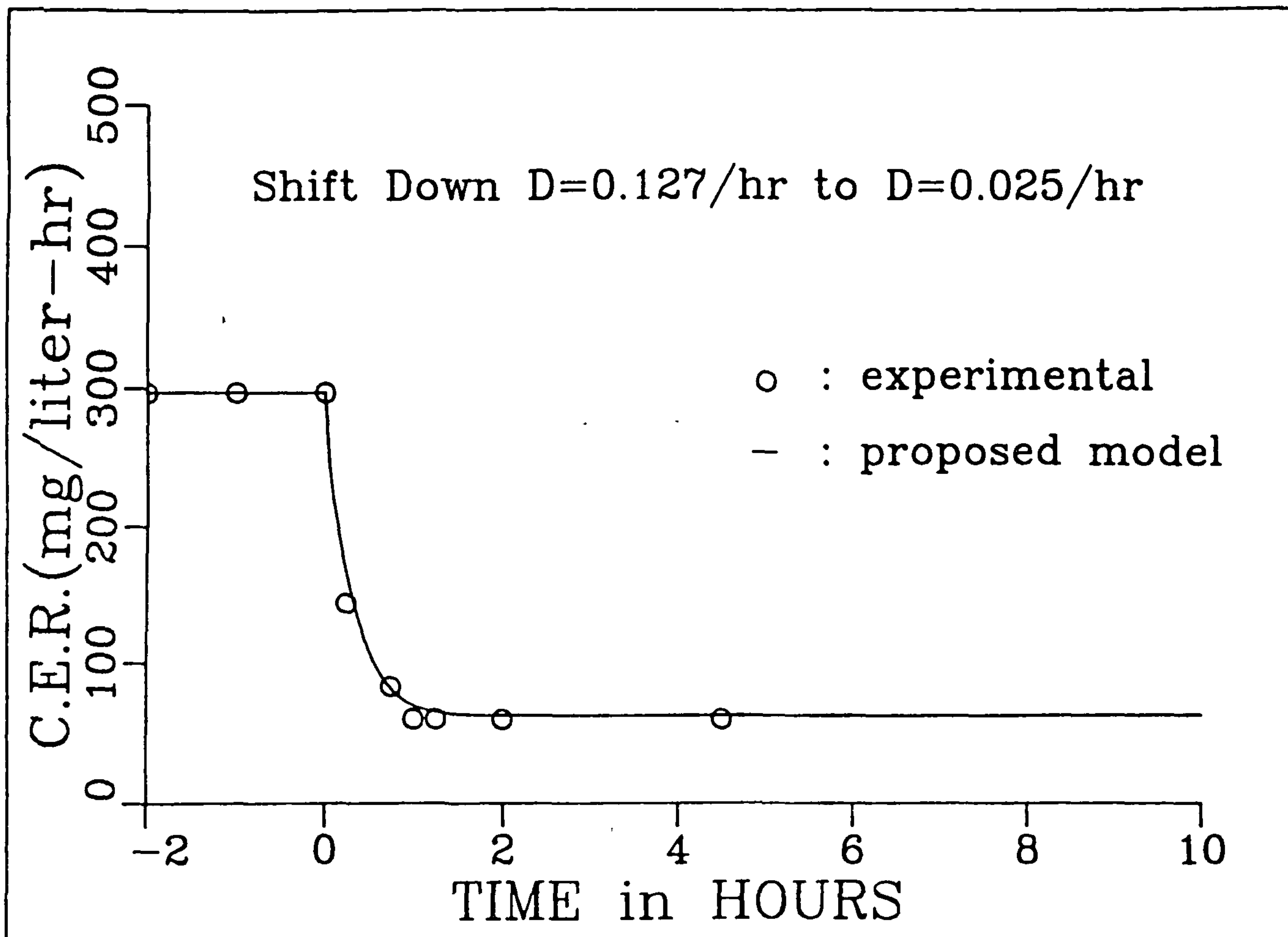

Figure 12. Comparison of CER data and model prediction of shift-down experiments.

been developed and are being verified experimentally.

SUMMARY

An experimental system was developed to study the microbial growth kinetic of an undefined mixed culture in an aerobic biological waste treatment process. The experimental results were used to develop a mathematical model that can predict the performance of a bioreactor. The bioreactor will be used to regeneratively treat waste material which is expected to be generated during a long term manned space mission. Since the presence of insoluble particles in the chemically undefined complex media made estimating biomass very difficult in the "real system", a "clean system" was devised to study the microbial growth from the soluble substrate.

A carbon mass balance was used to estimate microbial biomass. The CO_2 evolution rate(CER) was used to check the consistency of the carbon balance experimental results. CER can also be used to estimate biomass directly once Pirt's relationship with maintenance energy coefficients is known for a given system. The advantage of using CER is in its speed and precision. CER has been

demonstrated to be a reliable, reproducible, and sensitive indicator of biochemical state of a microbial system. Since CER can be monitored continuously on-line it can be used to control the biochemical reactor.

A mathematical model of microbial growth originally suggested by Andrews and Tien(1977) has been extended to study the chemostat processes. The model predictions have been experimentally verified both for steady state and for transient conditions using the simulated "clean system". The most important result of this investigation was that the model can predict the transient response of CER measurements very well without the use of adjustable parameters.

The use of the "clean system" allows us to check the validity of carbon balance - CER method of biomass estimation and to test the predictability of the model more readily. The microbial growth model developed from the "clean system" then can be modified by including the hydrolysis kinetic model of lignocellulosics to describe the "real system". The overall model for the "real system" and its experimental verification will be presented in a future report. Such a model will be useful in aiding NASA planners to evaluate the feasibilty

of incorporating the aerobic biological waste treatment system as a component of CELSS.

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