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Yeast-activated sludge model for aerobic degradation of a non-fermentable substrate

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ABSTRACT

This paper uses a combination of models and unique features not found in other models to develop a yeast-activated sludge model for modeling yeast kinetics while degrading an aerobic, non-fermentable substrate. Respirometry is used in conjunction with carbohydrate testing to determine model parameters and kinetics for yeast-activated sludge consuming glycerol at 30 g/l salinity. The data and model show that yeast metabolism begins with exponential growth similar to bacteria. After a minimum substrate level is reached, metabolism switches to storage/consumption of stored material. This phase is followed by a decay phase that is characterized by consumption of a portion of the material stored in the yeast cells. Values for kinetic parameters of the model are provided after fitting the model to four different substrate concentrations. Through the model, the differences between operation of bacterial and yeast systems become apparent and are explained.

Keywords: Yeast; Respirometry; Kinetics; Wastewater treatment; High salinity wastewater treatment

1. Introduction

Biological treatment of wastewater employs an assortment of technologies that are mature, well studied, and supported by years of operational data. Some wastewaters are difficult and expensive to treat either biologically or biologically in conjunction with physiochemical pre or post treatment. These hard to treat types of wastewater are primarily produced by industrial operations. The conditions of these wastewaters that inhibit biological treatment include high salinity, low pH, high organic load, high ammonia load, high temperature, and the presence of toxic substances. Yeast have been proposed and researched as a potential organism to treat some of these types of wastewater.

While research related to yeast treatment of wastewater is not voluminous, it is also not insignificant. The research generally focuses on a single yeast species as a treatment organism for a specific wastewater or contaminant. These research papers sometimes present yield (Y_{OHO}) and maximum substrate utilization values ($\mu_{OHO,Max}$). However, there is little data that cover all parameters that would be necessary to design and operate a yeast-activated sludge (YAS) treatment process. Also, there has never been a model proposed for use in evaluating, optimizing, and operating a YAS system.

The purpose of this paper is to present a model to be used for the determination of kinetic parameters when

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using yeast to treat wastewater aerobically, without fermentation. The model can be applied for determination of intrinsic yeast kinetics, extant yeast kinetics, operation of YAS systems, and optimization of YAS systems. The model is restricted to determination of kinetics and decay parameters within 3 d of feeding. Sludge activity for longer periods requires additional research due to the complex nature of yeast stationary phase activity.

1.1. Yeast as a wastewater treatment organism

Yeasts are single-celled fungi in the classes of *Ascomycetes* and *Basidiomycetes*. As fungi, they have inherent traits that make them potentially attractive for use as a waste treatment microorganism. They can tolerate a wide range of pH (2–9) compared to bacteria that generally require pH to be between 6 and 8 [1]. Yeast cells are more osmotolerant than bacterial cells [2]. Yeast have a high tolerance for both low nitrogen and high ammonia environments [1,3,4]. Yeast can tolerate very high COD/BOD loading rates in comparison to bacteria [3,5–7]. Despite these advantages, yeast have not been used to any great extent as the primary treatment organism because under normal conditions (pH ~7 and 15–20˚C) bacteria are faster at removing COD and BOD, generally easier to settle, dominant in terms of process biomass, and readily available (for seeding purposes) in the raw wastewater.

1.2. Yeast metabolism and substrate consumption

The primary substrate of choice for yeasts is glucose. All yeast species can use glucose as a substrate [8]. Other types of carbon sources that may be used by yeast are varied. If glucose is not the primary substrate, yeast must either convert the carbon source into glucose or an intermediate substrate that fits into the glycolytic cycle at some point [9]. Generally, the yeast metabolic cycle begins with glucose going through glycolysis. This process is the same for almost all yeast species except for species who use an alternate pathway within glycolysis called the pentose phosphate pathway [10]. Regardless of the pathway through glycolysis, about the same amount of energy is derived and the end product is pyruvate.

When substrate levels are high, the yeast multiply as fast as possible. As substrate levels begin to decline, they begin to turn substrate into storage products. Zakrajsek et al. [11] noted that even after a month with no substrate, the amount of glycogen in yeast cells did not decline. They suggested that yeast began production of triglycerols as primary substrate began to diminish. Subsequent consumption of these triglycerols may be the energy source used by yeast to remain viable for long periods with no substrate. After substrate in the bulk liquid becomes depleted, the cell cycle is suspended until substrate availability is restored [9]. Suspension of the cell cycle means that no new cells are produced and the cells enter the "stationary phase". During the stationary phase, stored substrate is slowly consumed at a rate just sufficient to keep the metabolic process going.

When discussing bacterial activated sludges, the phases of growth that the biomass goes through are: lag, exponential growth, stationary, and decay. When almost all substrate is consumed, the biomass enters the stationary phase. This phase does not last long. "Decay" or "endogenous respiration" follows. In the yeast literature, the stationary or "quiescent" phase is discussed extensively, but no mention is made of a decay phase. The lack of attention paid to the decay phase in yeasts most likely stems from the fact that the decay rate for bacterial sludge mass is relatively fast and has a typical value of 0.2 d^{-1}. In yeast cultures, greater than 90% of yeast cells will remain viable after 8 d (decay rate of 0.0125 d^{-1}) [11]. For periods that last a month without substrate addition, losses may be between 40 and 50% [11,12]. Zakrajsek et al. [11] showed that decay occurs in spurts. Minard and McAlister-Henn [12] found that there was 50% loss of viable biomass after 22 d.

1.3. Yeast-activated sludge model (YASM)

For this research, a combination of the activated sludge models (ASM) ASM1, ASM2, and ASM3 plus items taken from other models and additions unique to the YASM model were used. The incorporation and integration of so many methods into the model is mainly due to the ability of yeast to store carbohydrate and consume it when primary substrate is exhausted. An initial model based on ASM1 was prepared but after respirometric data were analyzed, it was discovered that the ASM1 model is not well suited to modeling the whole respirometric curve. ASM2 and ASM3 have features that can deal with decreasing rate of substrate consumption when substrate becomes limited. Therefore, YASM was constructed using the relevant and useful parts selected from among ASM1, ASM2, and ASM3. Additional modeling controls not included in these models was also used.

2. Methods

2.1. Enrichment and development of yeast biomass

The yeast sludge was developed using a combination of yeast from soy sauce manufacturing, alcohol

manufacturing, and breadmaking. Soy sauce yeast were obtained from bean paste out of a soy sauce fermenter prior to pressing for soy sauce extraction. Alcohol yeast were obtained from alcohol starter. Bread yeast were obtained in store-bought yeast packets.

The enrichment process for all yeast sources was performed as described in Standard Methods [13]. The pH of the enrichment reactors was kept close to 4.

Carbon source during initial enrichment was glucose. Difco yeast nitrogen base with amino acids was used as the nutrient source. Enrichment nutrient broth was composed of 10 g glucose and 5.6 g yeast nitrogen base per liter of solution. Following enrichment, salinity in reactors was gradually increased to 30 g/l over a period of two weeks.

After cultures were enriched and acclimated to high salinity, carbon source was changed to glycerol and nutrient feed was adjusted.

Final nutrient mixture was fed per 3,000 mg of new cell COD (not mg of cell biomass) grown is presented in Table 1.

2.2. Curve fitting

Berkeley Madonna was chosen for modeling kinetics in this research.

2.3. Respirometry

Respirometry was performed using a Challenge model AER-204 respirometer. Respirometry tests were all done at a starting pH between 5.0 and 5.1. Temperature of the reactors was controlled at 25˚C by a PolyScience model 5250M40A130Y refrigerated recirculating chiller. The curves shown in this paper were determined using a synthetic wastewater composed of glycerol, nutrient, NaCl, and phosphorus buffer. NaCl concentration was 30 g/l for all tests. Nutrient composition was the same as described in Table 1. Dosing of nutrient was determined by estimating a yield of 0.6 from glycerol COD and then adding a nutrient dose of 5x stoichiometric need.

Table 1
Feed water nutrient composition

Component	Mass (mg)
$(NH_4)_2SO_4$	857
KH_2PO_4	235
$MgSO_4{\cdot}7H_2O$	467
$ZnSO_4{\cdot}7H_2O$	0.5
$CaCl_2$	1
$MnCl_2$	1
$FeCl_2$	1
$(NH_4)_6M_7O_{24}{\cdot}4H_2O$	0.2
$CuSO_4$	0.2
$CoCl_2$	0.2
Yeast extract	94

Note: Components in Table 1 were used per 3,000 mg of new cell COD grown.

2.4. COD testing

COD of influent wastewater for both reactors and respirometry bottles was tested after the addition of glycerol and nutrient, but prior to the addition of NaCl so that chlorine interference would not be a factor. COD tests were carried out as described in Standard Methods 5220 B [13]. When testing high salinity samples, EPA method 410.3 was used.

2.5. Carbohydrate and stored material tests

Carbohydrates stored in the cells as well as carbohydrates in the bulk liquid were determined by the anthrone test as described by Jenkins et al. [14].

Carbohydrate testing was performed on raw sludge, washed cells, and supernatant. To separate and wash cells, raw sludge was centrifuged at 10,000 g for 3 min in a model H-1650-W centrifuge made by Xiang Yi. Supernatant was poured off and tested for carbohydrates. Deionized water was added to the centrifuge tube to the volume prior to decanting supernatant. Cells were re-suspended using a Qilinbier model QL-866 vortex shaker. The re-suspended cells were then tested for carbohydrate content.

Spectrophotometry for the test was performed using a PGeneral model 19-1650-01-0208 spectrophotometer.

2.6. Genetic testing

Biomass samples were freeze dried and sent to Novogene in Beijing. Genetic material was extracted and run through an ITS1 indexing process to determine the genetic type and proportion of fungal organisms in the sludge.

3. YASM description and explanation

3.1. Model assumptions

The yeast YASM model used in this research includes the following assumptions:

(1) The kinetics modeled are only accurate for the first three days after feeding. Beyond that point, stationary stage activity of the yeast biomass is complex and is not included in the model.

(2) Yeast kinetics can be modeled in part by Monod–Herbert kinetics.
(3) Because the pH of the respirometry experiments was 4.9–5.1 and the fact that yeast do not nitrify, it is assumed that bacterial nitrifier activity was either completely stopped or inhibited to the point that their action could be ignored.
(4) Endogenous decay as proposed in the ASM3 model was used instead of the death/re-growth concept of the ASM1 model.
(5) Heterotrophic growth, respiration, and yield occur as modeled by both the ASM1 and ASM2 models.
(6) Hydrolysis can be modeled as proposed by the ASM3 model.
(7) A portion of carbohydrates stored in the cells is in the form of glycogen or integral cell material (such as the cell wall) and will not be consumed by the biomass in the time period considered by the model (within 3 d of feeding).
(8) Degradation of $X_{B,Stor}$ occurs according to a first-order rate constant in the same way as overall cell decay.

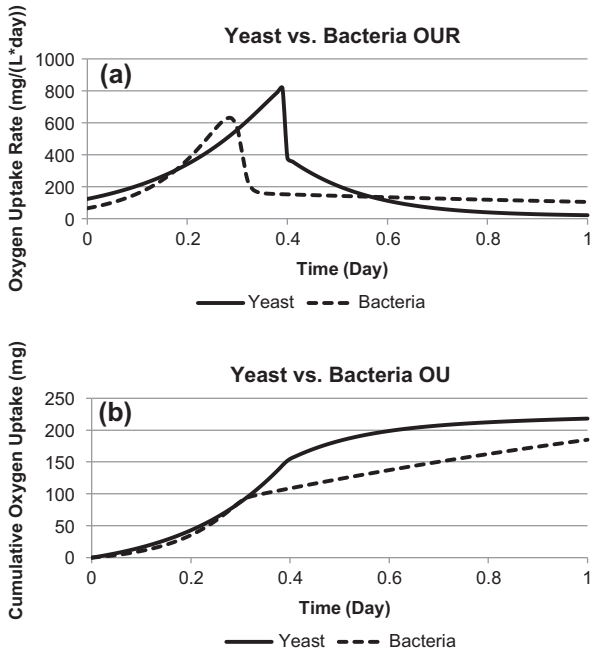

Fig. 1. (a) Top shows oxygen uptake rate (OUR) curves for yeast and bacteria and (b) bottom shows the oxygen uptake (OU) curves for the same conditions.

3.2. Yeast vs. bacterial respirograms

Yeast respirograms differ from bacterial respirograms. The reason for creating the YASM model was due to these differences. Fig. 1(a) shows a yeast oxygen uptake rate curve (OUR) curve compared to a bacterial OUR curve. Fig. 1(b) shows the corresponding oxygen uptake curves (OU). The curves were constructed using the YASM model for yeast and a typical ASM1 curve for the bacteria. Kinetic values used for yeast were determined from an actual respirometric test with the YAS consuming 600 mg/l glycerol at 30 g/L NaCl. Kinetic values for bacteria are kinetic values for bacterial degradation of carbohydrates suggested by Rittman and McCarty [1]. Some kinetic values for the bacterial curve such as decay rate were supplemented from the ASM1 model where carbohydrate kinetics could not be found [15]. Substrate concentration and initial biomass concentration was modified so that it was the same for both models.

Fig. 1 shows that the initial yeast consumption of substrate is slower than bacteria. The bacterial curve quickly peaks and then drops to endogenous respiration. The yeast have a longer, slower increase in rate then a sharp decline. The fall in oxygen uptake rate after the peak for bacteria falls all the way to their final endogenous respiration rate. The yeast curve falls to a point fairly far above the final endogenous respiration rate and then gradually declines. The final endogenous respiration rate for yeast is lower than that of bacteria.

The ASM1 model can be fit to the exponential portion of the yeast curve, but does not fit the curve after the peak. The yeast curve after the peak looks very similar to what can be modeled using ASM2 or ASM3 for stored substrate consumption, but those models do not fit the exponential portion of the curve meaningfully.

3.3. YASM model

The proposed YASM model is shown in Table 2. The table is set up in the ASM1 style. A written statement of the model is as follows: When substrate is plentiful, the yeast grow according to ordinary Monod kinetics. They go through the lag and exponential growth phase the same as a bacterial aerobic sludge mass. During this phase, decay of cell mass is determined by the death rate of cells. Yeast cells die when they can no longer divide and form new cells. At some point $K_{I,SB}$, substrate becomes limited and the yeast suspend the cell cycle. After this time no new cells are formed and all substrate is packaged as storage material $X_{B,Stor}$. As substrate runs out and becomes less in relation to cell mass, conversion of

Table 2
YASM model

	i	1	2	3	4	5	6	7	8	9	10	11	Process rate
j	Process	S_U	S_B	$X_{B,Stor}$	X_U	X_{CB}	X_{OHO}	S_{O2}	S_{NH4}	X_{UNH4}	S_{PO4}	X_{UPO4}	
1	Aerobic growth of yeast on Sa		$-1/Y_{OHO}$				1	$1-1/Y_{OHO}$	$-i_{N\_OHO}$		$-i_{P\_OHO}$		(if $S_B > K_{I,SB}$ then 1 else 0) × $\mu_{OHO,MAX}$ × X_{OHO} × (S_B/($K_{S,OHO}$ + S_B))
2	SMP production during exponential growth	Y_{SB_SU}	$-Y_{SB\_SU}$										(if $S_B > K_{I,SB}$ then 1 else 0) × $\mu_{OHO,MAX}$ × X_{OHO} × (S_B/($K_{S,OHO}$ + S_B))
3	Storage phase		$-1/Y_{SB\_Stor}$	1				$1-1/Y_{SB\_Stor}$					(if $S_B > K_{I,SB}$ then 0 else 1) × $\mu_{OHO,Stor}$ × X_{OHO} × (S_B/X_{OHO})/($K_{S,OHO,Stor}$ + (S_B/X_{OHO}))
4	Cell mass decay, exponential growth				f_{XI}		−1	$-(1-f_{XU\_OHO,lys})$	i_{N_OHO} × $(1-f_{XU\_OHO,lys})$	i_{N_OHO} × $f_{XU\_OHO,lys}$	i_{P_OHO} × $(1-f_{XU\_OHO,lys})$	i_{P_OHO} × $f_{XU\_OHO,lys}$	(if $S_B > K_{I,SB}$ then 1 else 0) × $b_{OHO,Exp}$ × X_{OHO}
5	Cell mass decay, non-exponential growth				f_{XI}		−1	$-(1-f_{XU\_OHO,lys})$	i_{N_OHO} × $(1-f_{XU\_OHO,lys})$	i_{N_OHO} × $f_{XU\_OHO,lys}$	i_{P_OHO} × $(1-f_{XU\_OHO,lys})$	i_{P_OHO} × $f_{XU\_OHO,lys}$	(S_B × ≤ $K_{I,SB}$ then 1 else 0) × $b_{OHO,Stor}$ × X_{OHO}
6	Aerobic degradation of X_{sto}			−1				−1	$-i_{N\_OHO}$		$-i_{P\_OHO}$		(if $S_B \geq 0$ and $S_B \leq K_{I,SB}$ then $(1-S_B/K_{I,SB})$ else 1) × b_{Stor} × $X_{B,Stor}$
7	SMP production from degradation of stored substrate	$Y_{XB,Stor\_SU}$	$-Y_{XB,Stor\_SU}$										(if $S_B \geq 0$ and $S_B \leq K_{I,SB}$ then $(1-S_B/K_{I,SB})$ else 1) × b_{Stor} × $X_{B,Stor}$
8	Hydrolysis of X_{CB}	$f_{SU\_XCB,hyd}$	$1-f_{SU\_XCB,hyd}$			−1			i_{N_XCB} × $(1-f_{SU\_XCB,hyd})$	i_{N_XCB} × $f_{SU\_XCB,hyd}$	i_{P_XCB} × $(1-f_{SU\_XCB,hyd})$	i_{P_XCB} × $f_{SU\_XCB,hyd}$	$q_{XCB,hyd}$ × X_{OHO} × (X_{CB}/X_{OHO})/($K_{S,OHO,hyd}$ + (X_{CB}/X_{OHO}))
Rate name		Inert substrate (mg COD/L d^{-1})	Readily degradable substrate (mg COD/L d^{-1})	Stored material (mg COD/L d^{-1})	Inert material from biomass (mg COD/L d^{-1})	Hydrolysable substrate (mg COD/L d^{-1})	Active biomass (mg COD/L d^{-1})	Oxygen consumption rate (mg O_2/L d^{-1})	Readily consumable nitrogen in substrate (mg N/L d^{-1})	Inaccessible nitrogen in substrate (mg N/L d^{-1})	Readily consumable phosphorus in substrate (mg N/L d^{-1})	Inaccessible phosphorus in substrate (mg N/L d^{-1})	

substrate to storage products slows down. Concurrently, the consumption of stored materials begins. Energy solely from the consumption of stored substrate reaches its maximum rate when all primary substrate is consumed. Once the storage phase is reached, decay rate is composed of two parts. The first part is the decay of stored material which is relatively rapid. The second part is endogenous decay of biomass which is relatively slow while stored material remains.

This model necessarily simplifies many complex processes. For instance, the carbohydrate tests suggest that some storage occurs during the cell growth phase. The carbohydrate tests also showed that some of the stored material is initially consumed immediately after feeding. Modeling these complex behaviors would require additional but unnecessary model complexity. Output from the current model mimics the overall process well.

Inert nitrogen and inert phosphorus rates for X_{OHO} and X_{CB} have been added to the table in a manner similar to that used in ASM3 except that conversion of organic nitrogen to ammonia is not included. Yeast can use a number of nitrogen sources, but their ability to convert organic nitrogen to ammonia nitrogen is unknown at this time. The model assumes that all usable nitrogen is captured in the S_{NH4} term. The coefficients for X_{OHO} nitrogen and phosphorus content are based on the YAS cell composition research of a number of different yeast sludges grown under a variety of conditions (research not presented here). The result of that research was an empirical formula for yeast cells: $C_{7.1}H_{12.7}O_{3.7}NP_{0.08}$. The proportion of phosphorus and nitrogen in this empirical formula agrees well with that from other research [5,6]. This empirical formula has a g cell COD/g cell ratio of 1.44.

Table 3 defines the symbols and units used in Table 2. Table 3 also provides values that were determined based on respirometry and carbohydrate tests. The values derived from the respirometry tests are average values from the fitted curves of YAS consuming four different concentrations of glycerol. Each concentration was tested in duplicate.

3.4. Explanation of model equations, parameters, and variables

Many of the components of YASM are similar to the ASM1, ASM2, and ASM3 models and do not need further explanation. Some components differ from other models, however, and an explanation is provided below.

3.4.1. Yield

There are two yields for the model. One yield is for the phase when substrate is plentiful (Y_{OHO}) and one yield is for the storage phase when substrate becomes limited ($Y_{SB,Stor}$). The Y_{OHO} yield accounts for the cell biomass as well as the biomass glycogen stores. The $Y_{SB,Stor}$ is the stored carbohydrate that can be immediately used by the biomass when the primary substrate is exhausted.

3.4.2. $K_{I,SB}$

$K_{I,SB}$ is the inhibition constant. Once substrate reaches this concentration, yeast metabolism switches to storage metabolism. The use of this constant is similar to what Wayman and Tseng used [16]. It is an oversimplification. In a real system, there is not a complete and instantaneous shift from one type of metabolism to another. Earlier models produced by the research group captured this gradual shift with the help of some additional variables, but it was discovered that the meaning of the variables was hard to define. In addition, the rate that the biomass switches from one type of metabolism to the other was determined to be very fast, especially at high biomass concentrations. Simplifying by making a single cut-off value allows for more meaningful data as well as more convenient curve fitting.

The value $K_{I,SB}$ changes as biomass concentration changes. Due to this, $K_{I,SB}$ must be determined initially by curve fitting for each respirometry test. In order to calculate $K_{I,SB}$ during simulations, a formula was developed.

The carbohydrate tests allowed the research group to observe carbohydrate concentration in the cell mass over time. Fig. 2 shows the carbohydrate concentration in the cell mass over a 10 h period. Fig. 2 also shows COD concentration in the reactor over the same time period. Fig. 3 shows the carbohydrate and MLSS concentration over a 3 d period. From the data in Fig. 3, the carbohydrate decay rate was determined by taking the natural log of the decay rate, plotting it against time and finding the slope of the line. This is the same method used to determine endogenous decay rate described in ASM3 [15]. Using this decay rate, it is possible to calculate how much material is degraded and how much is left after that material has been consumed. The calculations show that (in Fig. 3) roughly 2,083 mg carbohydrate could be degraded at the rate calculated with about 1,000 mg carbohydrate remaining as stored carbohydrate and/or cell material. Fig. 2 shows that, after all substrate is consumed, the carbohydrate contained in the cells is about 3,800 mg/l. Therefore,

Table 3
YASM model variable meaning, units, and values obtained during this research

Compounds	Symbol meaning	Unit	Value
S_U	Non-biodegradable soluble COD	mg COD/L	
S_B	Readily degradable substrate	mg COD/L	
$X_{B,Stor}$	Stored substrate	mg COD/L	
X_U	Inert biomass	mg COD/L	
X_{CB}	Hydrolysable substrate	mg COD/L	
X_{OHO}	Active biomass	mg COD/L	
S_{O2}	Oxygen consumption	mg O_2/L	
S_{NHx}	Wastewater nitrogen content including all usable nitrogen sources	mg N/L	
$X_{U,NHx}$	Inert nitrogen	mg N/L	
S_P	Wastewater phosphorus content	mg P/L	
$X_{U,P}$	Inert phosphorus	mg P/L	
Kinetic parameters			
Y_{OHO}	Yield for ASM1phase consumption of substrate	g YAS cell COD/g S_B	0.49
$Y_{SB,Stor}$	Yield from storage of substrate	g $X_{B,Stor}$ COD/g S_B	0.45
Y_{SB_SU}	SMP formed during primary substrate consumption	g SMP COD/g S_B	0.01
$Y_{XB,Stor\_SU}$	SMP formed during stored substrate consumption	g SMP COD/g $X_{B,Stor}$	0.2
$\mu_{OHO,Max}$	Max utilization rate for ASM1 phase consumption of substrate	d^{-1}	5.44
$\mu_{OHO,Stor}$	Max utilization rate for conversion of substrate to $X_{B,Stor}$	d^{-1}	13.14
$K_{S,OHO}$	Half saturation constant for ASM1 phase S_B	mg S_B COD/L	15.25
$K_{S,OHO,Stor}$	Half saturation constant for conversion of substrate to $X_{B,Stor}$	mg S_B COD/L	3.59
$K_{S,OHO,hyd}$	Hydrolysis saturation constant for X_{CB}	g XC_B COD/(g X_{OHO} COD d^{-1})	
$K_{I,SB}$	Inhibition constant for storage phase	mg S_B COD/L	
$b_{OHO,Exp}$	Biomass decay coefficient, exponential growth phase	d^{-1}	0.035
$b_{OHO,Stor}$	Biomass decay coefficient, storage and stationary phases	d^{-1}	0.0125
b_{Stor}	$X_{B,Stor}$ decay coefficient	d^{-1}	0.64
$i_{N,OHO}$	Nitrogen content of X_{OHO}	g N/g X_{OHO} COD	0.06
$i_{N,XCB}$	Nitrogen content of X_{CB}	g N/g XC_B COD	
$i_{P,OHO}$	Phosphorus content of X_{OHO}	g P/g X_{OHO} COD	0.01
$i_{P,XCB}$	Phosphorus content of X_{CB}	g P/g XC_B COD	
$f_{XU\_OHO,lys}$	Fraction inert material from endogenous respiration	g X_U COD/g X_{OHO} COD	
$f_{SU\_XCB,hyd}$	Fraction inert material from hydrolysis of X_{CB}	g S_U/g XC_B COD	

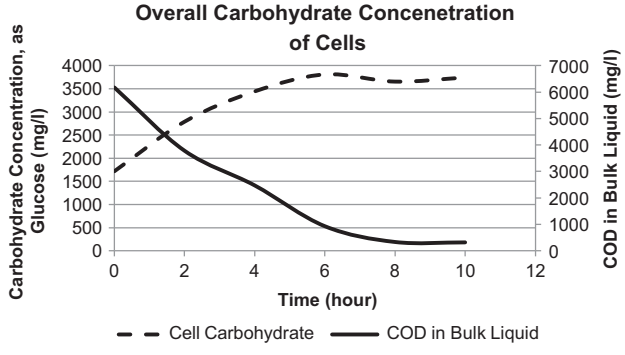

Fig. 2. Plots the change in COD in the reactor simultaneously with biomass carbohydrate concentration change.

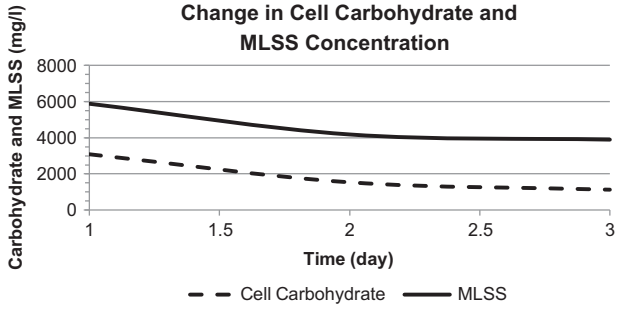

Fig. 3. Plots the change in carbohydrate concentration in the MLSS and the change in MLSS over a 3 d period.

the total carbohydrate concentration in the cells that is available for immediate degradation after primary substrate is exhausted is about 2,800 mg/l. By using the amount of stored material calculated in conjunction with biomass concentration, we calculated the percentage of carbohydrate stored in the cell mass. This value

in conjunction with a decay rate determined by the kinetic experiments was used in the model to simulate batch reactor operation. The results fit well to what was observed in the reactors.

Additional evidence for the decay of a stored substrate comes from the b_{Sto} values. b_{Sto} values, which were determined in the kinetic tests and averaged 0.64. Prior to that, b values were calculated from numerous endogenous decay respirograms and those decay values were always between 0.4 and 0.8. Such high decay values are unlikely to be due to death of biomass. Decay (death) for bacteria is typically given between 0.1 and 0.2 1/d [1,17,18]. Yeast decay rate, especially during the first 10 d after feeding, is very slow and so the high degradation rate could not be from cell decay [11]. The amount of carbohydrate determined through the carbohydrate testing fits with the fast decay rate that was seen in the endogenous decay respirograms. That is, fast decay rate was due to biomass consumption of stored material and not due to cell death.

Decline in mass of stored carbohydrate due to consumption by biomass was almost 1:1 with decline in MLSS concentration as can be seen in Fig. 3. MLSS concentration at 10 h after feeding was 6,400 mg/l. Since about 2,800 mg/l is stored carbohydrate, the cell mass is 44% consumable carbohydrate upon depletion of primary substrate.

Through knowledge of the required carbohydrate percentage prior to exhaustion of primary substrate as well as knowing cell COD/Cell mass = 1.44, we can calculate $K_{I,SB}$ for any concentration of biomass by using Eq. (1):

$$K_{I,SB} = \frac{\left(\frac{X_{OHO}}{1.44} \times 0.44 - X_{B,Stor}\right)}{Y_{SB,Stor}} \quad (1)$$

This equation bases the storage phase of the biomass on the amount of stored material they already contain as well as the amount that they must contain upon consumption of all primary substrate.

Eq. (1) cannot be applied to curve fitting and is only to be used for simulations of a mature sludge. This is due to the fact that it takes some time for the biomass to reach its maximum $X_{B,Stor}$ concentration. After a single respirometry run, the stored carbohydrate concentration as determined by the anthrone test is only around 20%. This value is similar to what is returned if MLSS and carbohydrate percentage are calculated from the fitted respirograms. Once the reactors have operated for some time and have a stable biomass concentration, they will reach their maximum storage of 44%. In this research, the average percentage of total carbohydrate in the cell 24 h after feeding the mature reactors was 57%. Zheng et al. [19] found a carbohydrate content of 55% in their yeast biomass. It was not reported in that research when the sample was taken. The similarity in storage may not be coincidental as the biomass in that research was composed entirely of *Candida utilis* which is a synonym for the primary population of yeast in this research *Cyberlindnera jadinii* (see Section 4.2).

3.4.3. Conditional statements

In the ASM models, saturation or inhibition equations are typically used to control various microbial actions. Yeast, however, are hard to model using only saturation and inhibition equations. Conditional statements are easier to use in some circumstances. The conditional statements included in the model are described below:

(1) If $S_B > K_{I,SB}$ then 1 else 0: this turns on and off all processes related to the growth phase metabolism (when $S_B > K_{I,SB}$). This includes growth of cell mass, decay of cell mass at growth phase rate, and production of soluble microbial products (SMP) at growth phase rate.
(2) If $S_B > K_{I,SB}$ then 0 else 1: This turns on and off all processes related to storage and stationary phase metabolism (when $S_B < K_{I,SB}$). This includes storage of substrate, decay of cell mass at storage phase rate, and production of SMP at storage phase rate.
(3) If $S_B \geq 0$ and $S_B \leq K_{I,SB}$ then $(1 - S_B/K_{I,SB})$ else 1: this conditional statement controls the slow startup of consumption of stored material after storage phase has begun.

3.4.4. $b_{OHO,Exp}$, $b_{OHO,Stor}$, and b_{Stor}

Decay in yeast is complicated due to their ability to store and consume stored material. Multiple decay rates are needed. The $b_{OHO,Exp}$ parameter applies to the yeast during the exponential growth phase. This value was not directly calculated from the respirogram. It is derived from general knowledge of yeast behavior. Under normal circumstances and in absence of external factors, yeast life span is governed by the number of cell divisions they have. After every cell division, the cell forms a region of scar tissue that can no longer be used to produce daughter cells. Once the cell surface is completely covered with this scar tissue, it can no longer bud and dies [9]. Typical number of

divisions is 13–30 before no more divisions are possible [9]. Doubling time for *Saccharomyces cerevisiae* time under ideal conditions is about 1.5 h [17]. Based on this, a decay rate of 0.035 1/d was calculated. It is unlikely that conditions in the reactor would allow division at such a high rate continuously. For instance, there is a lag phase after substrate is added to the reactor that would result in a cell division rate far less than the maximum. Effects of temperature, aeration, pH, salinity, etc. would also affect cell division rate to some degree causing it to be less that optimal. Conversely, there are stresses on the cells that may make them decay faster such as oxidants. Since it is difficult to decide what value is most appropriate, the research group decided simply to use 0.035 as the $b_{OHO,Exp}$ value with the knowledge that this value is only a rough approximation.

The $b_{OHO,Stor}$ decay rate applies to decay during the period of stored material consumption. The derivation of this rate was described in Section 1.2 and has a value of 0.0125 1/d.

The b_{Stor} term governs the decay of stored material. This rate was determined during curve fitting and many endogenous decay tests. This rate only applies while there is still quickly degradable stored material in the cell (about 3 d). After readily degradable stored material is all consumed, the decay rate of cells changes and is not included in this model.

3.4.5. $Y_{SB,SU}$, $Y_{XB,Stor,SU}$

Activity of the biomass in degrading the primary substrate produces SMP that cannot be easily degraded (S_U). Some models include a term for calculation of this material [1,20]. Smetts gives a value of 0.05 g SMP COD/g COD while Rittman and McCarty give a provisional value of 0.12 g SMP COD/g COD degraded. In the ASM models, SMP is only partially accounted for as the portion defined by Rittman and McCarty as biomass associated products (BAS). The ASM models do not include what Rittman and McCarty define as substrate utilization associated products (UAP). Decay of biomass is very slow in the yeast systems as described in Sections 1.2 and 3.4.5. Therefore, BAS production is low. However, there is significant SMP produced during the treatment process, especially during the stationary phase while the yeast are consuming stored material. This UAP SMP was measured both by COD and carbohydrate tests.

The research group used the COD and carbohydrate tests shown in Figs. 2 and 3 and others to determine how much SMP was produced during different phases of substrate degradation. These values were converted into yield of SMP for primary and stored material degradation ($Y_{SB,SU}$, and $Y_{XB,Stor,SU}$, respectively). The value determined for $Y_{SB,SU}$ was 0.01 which is lower than what has been attributed to bacterial sludges. The value determined for $Y_{XB,Stor,SU}$ was 0.2 which is a bit higher than for bacterial sludges. The actual SMP produced in each of these phases varied somewhat with time and the yields are overall values for the whole of each phase.

3.4.6. Calculating MLSS

The X_{OHO} calculated by the model is in terms of cell COD. In order to determine actual cell mass, this value must be divided by 1.44. The biodegradable carbohydrate content was essentially 1:1 MLSS to mass determined. Therefore, MLSS can be calculated from the model by Eq. (2):

$$\text{MLSS} = \frac{X_{OHO}}{1.44} + X_{B,Stor} \quad (2)$$

4. Results and discussion

The following is a review of the results as well as discussion about select aspects of the model.

4.1. Yeast selection and culture development

It is well known that a mixed population of microorganisms is beneficial for the operation of a wastewater treatment plant. The reason for this is that a sludge composed of a mixture of organisms is more adaptable to changes in influent wastewater composition. Dan used mixed YAS in his research [5,18] as did Zheng et al. [19,21]. Several mixed yeast cultures were chosen for use in this research. The original yeast seed sludge came from the unprocessed beans in a soy sauce fermentation tank in Guangzhou China. These yeast were preliminarily chosen because they are fermented in high salinity, high organic loading conditions. It was surmised that they would be very osmotolerant and well suited for treatment of saline wastewater.

The enriched yeasts were tested for ability to degrade of a variety of industrial wastewaters. It was discovered during testing that the acid producing/acid tolerant properties of some yeast strains was useful for the treatment of some specialized wastewaters. Thereafter, other yeast cultures were raised. These included typical bread yeast, yeast used to make a

Chinese liquor called "baijiu" and yeast used to ferment another type of Chinese alcohol known as "mijiu." The baijiu and mijiu yeast are both mixed cultures developed by Chinese farmers who make the alcohol starter.

A number of carbon sources were investigated for suitability as a non-fermentable substrate for this research including: glucose, glycerol, acetate, ethanol, methanol, and several industrial wastes. The object was to determine which substrate produced the "cleanest" reaction for kinetic testing. Ethanol was found to produce the best curve but at high doses it appeared to inhibit growth of the biomass. The respirogram produced during glycerol breakdown was also very good and lead to glycerol being selected to be the primary substrate. The nutrient solution that was added to the carbon source was based on the nutrient mixture used by Dan in his research but the nitrogen and phosphorus content were modified [5]. The modification in these nutrients was due to data concerning yeast cell composition that was developed during the authors' research (research not presented here). Also, dosing of the nutrient was not based on wastewater COD but on growth of new cell COD/d. COD of cells grown each day was initially estimated by the YAS VSS content but later was based on the YASM model. The reason for this deviation from the way nutrient is typically fed is due to the way yeast biomass grows. When the biomass is young and growing rapidly, more nutrient is needed because it is used in the new cell mass. As the biomass becomes mature, daily cell production declines and the nutrient dose needs to be reduced for fill and draw reactors. If the nutrient is not reduced, ammonia builds up to critical levels that will eventually kill the biomass. Once the model was developed, it was very easy to see how much biomass was being produced per day and adjust nutrient dosing accordingly. Prior to completion of the model, many reactors were killed at about three weeks due to excessive ammonia concentration. This dosing requirement is not necessary for batch reactors unless the operator wants to reduce chemical usage.

4.2. Genetic testing results

Identification of the species in the sludge was not the purpose of this research but the research group felt that identification might prove useful for comparison with other sludges at a later date. There were 43 species of fungi present in this sludge. The top five species and their proportions in the sludge are shown in Table 4.

It is noteworthy that other sludges running on different substrates and under different salt concentrations had significantly different biomass profiles although most species were common to all cultures. This is to be expected as they were derived from the same parent sludge. However, the respirometry curves all have a similar shape to the ones from this reactor. This suggests that YASM is appropriate for a wide range of yeast species and may be extendable to other varieties of fungal species.

4.3. Respirometry results

Respirometry tests were run in duplicate at four different glycerol concentrations. The resulting curves were fitted to the model. Tests were done as described in Section 2.3. Fitted kinetic parameter values for all eight runs are presented in Table 5. Fig. 4 shows two examples of the model after fitting to the respirogram.

The model fits the data very well. Every run had a small amount of adaption at the beginning of the run which is clearly visible in the μ vs. OU curve was constructed as described by Smets et al. [20] for analysis of the exponential phase. The presence of an adaptation period is likely due to the pH during the tests. The reactor that the biomass was cultured in was run at a pH of ≤ 4.0 in order to inhibit bacterial growth. However, respirometry tests were run at pH of 5.0 because suitable inorganic buffer for pH 4.0 could not be found. A pH of 5.0 is at the very lowest end of the phosphate buffer's range. Large doses of buffer were needed and they still could not control the pH fluctuation completely.

The kinetics from curve fitting for most of the respirograms is consistent for most parameters. The 432 and 804 runs were performed at the same time while the 1,121 and 1,439 runs were done at the same time about a week later. Some of the variance in values (such as $K_{S,OHO}$) was no doubt due to the biomass characteristics changing slightly in the time between the respirometry tests.

Table 4
Sludge composition determined in DNA testing

Rank	Family and genus	% in sludge
1	*Cyberlindnera jadinii*	73.84
2	*Debaryomycetaceae Scheffersomyces*	15.62
3	*Trichomonoscaceae Zygoascus*	4.76
4	*Amanitaceae Amanita*	3
5	*Pichiaceae Pichia*	1.08

Table 5
Average results for variables after curve fitting to various influent glycerol concentrations

Name	432 A	432 B	804 A	804 B	1,121 A	1,121 B	1,439 A	1,439 B	Averages
Influent X_{OHO} (mg/l)	20	20	42	41	56	55	66	66	NA
Y_{OHO} (mg/l)	0.52	0.58	0.5	0.48	0.472	0.49	0.444	0.43	0.49
$Y_{SB,Stor}$ (mg/l)	0.46	0.49	0.38	0.4	0.45	0.4	0.49	0.49	0.45
$Y_{SB,SU}$	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
$Y_{XB,Stor,SU}$	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.20
$\mu_{OHO,Max}$	5.9	6.3	5.5	5.4	5.1	5.2	5.1	5	5.44
$\mu_{OHO,Stor}$	13	13.2	11.7	13.5	13.5	12.8	14	13.4	13.14
$K_{S,OHO}$ (mg/l)	11	11	10	8	22	20	20	20	15.25
$K_{S,OHO,Stor}$ (mg/l)	3.1	2.4	4	4.2	3.7	3.9	3.8	3.6	3.59
$K_{I,SB}$ (mg/l)	101	90	200	190	281	251	410	400	NA
$b_{OHO,Exp}$ (1/d)	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035
$b_{OHO,Stor}$ (1/d)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
b_{Stor}	0.62	0.55	0.68	0.67	0.64	0.58	0.68	0.71	0.64

Note: Results for kinetic parameters determined by curve fitting from various concentrations of glycerol a carbon substrate.

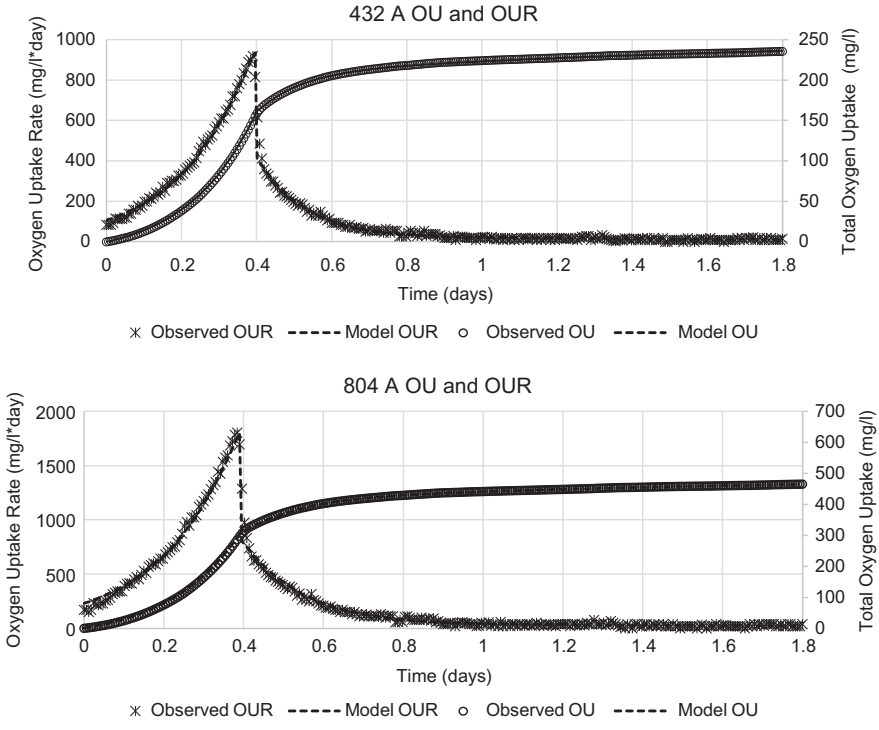

Fig. 4. Data from respirometry test (a respirogram) and the model output after curve fitting. Oxygen uptake rate (OUR) is plotted against the left *Y* axis. Total oxygen uptake (OU) is plotted against the right *Y* axis.

4.4. Existence and use of carbohydrates

The portion of carbohydrate that is consumable in the yeast cells is something that is unique to yeast and adds considerable complexity to the modeling of yeast. Decay rates from the endogenous decay portion of the respirograms are far too high to be associated with decay of biomass. It was observed that essentially all substrate was consumed by 8–10 h after feeding. At this point, the "decay" portion of the respirogram began. The decay rate in the respirograms was far too fast to be death of cell mass. At the rate observed, more than 50% of the cells would have died in the first day after feeding. The carbohydrate test revealed that there was, in fact, a substrate that was declining

in concentration (being consumed) at the rate being observed in the respirogram. This substance was the carbohydrate stored in the yeast biomass. MLSS tests were able to show a direct link between decline of the stored carbohydrate concentration and the mass of the MLSS.

4.5. Batch and continuous feed models

Reactors were operated using fill and draw method, batch operation, and continuous feed/discharge using a microfilter membrane. The fill and draw method involves only discharging the amount of mixed liquor required to meet the target SRT value. Nutrient and carbon source are added to the volume of water discharged and the reactor is refilled. Batch operation involves completely draining the reactor and separating the sludge from the effluent. The separated sludge is added back into the reactor with a new batch of wastewater. In continuous feed/discharge reactors, influent is slowly dripped into the reactor at a constant rate and removed at the same rate. This is typical of completely stirred tank reactor (CSTR) type operation. Due to poor settling characteristics of the yeast, a microfilter was used in the yeast CSTR to separate solids and supernatant.

The model was tested against and, in large part, calibrated by using the fill and draw and batch feed type reactors. Simulations were set up using Berkley Madonna and results were compared vs. daily MLSS and effluent COD tests. Values for kinetic variables used in the simulation were the average values from Table 5. $K_{I,SB}$ and MLSS were calculated as described in Section 3. Results from the simulation are given in comparison to both methods of operation in Table 6.

The model can be set up for CSTR operation simulation. However, we found that yeast are unable to remain dominant in CSTR reactors no matter how conditions are adjusted to favor yeast over bacteria (low pH, high salinity, high daily loading rate). The reason for this is explained in Section 4.6. Because a stable CSTR could not be established, the model output could not be compared to actual reactor results.

4.6. YASM implications for operation of a YAS system

YASM predicts the reaction outcomes of a YAS system well. The model also has other uses. The YASM provides useful guidance to facilitate the optimization and operation of a YAS system. The following four principles should guide yeast reactor operation and design.

4.6.1. High instantaneous loading is necessary

The growth curve for YAS is similar to that of a normal activated sludge up until the storage stage. At neutral pH and low salinity, yeast are unable to grow as fast as bacterial and are therefore a much smaller portion of the biomass (they have slower kinetics). However, if pH is low and/or other factors such as high salinity that favor yeast are present in the reactor, yeast kinetics are fast enough to allow them to compete with bacteria (kinetics are as fast or faster than bacteria). These fast kinetics only last as long as substrate loading is above $K_{I,SB}$. After substrate falls below this level, new yeast cells are no longer produced and increase in the mass of already existing cells slows as substrate becomes less available. Bacterial biomass, on the other hand, will continue to consume substrate and produce new cells as fast as possible until all substrate is consumed. Therefore, the longer the loading stays high, the longer yeast biomass will continue to produce new cells and the easier it will be to maintain a yeast dominated sludge.

In a typical CSTR, there is a large biomass and substrate is trickled in. The biomass grows to a size that essentially instantly reduces influent COD so that the instantaneous load on the reactor is always low no matter how high the daily load may be. This situation

Table 6
Comparison between results predicted in a simulation using the model and actual results from samples drawn from reactors

Operation type	Typical COD in effluent after 24 h	COD predicted by model at 24 h	Typical MLSS after 24 h (mg/l)	MLSS at 24 h predicted by model (mg/l)	Typical readily degradable carbohydrate at 24 h (mg/l)	Readily degradable carbohydrate at 24 h predicted by model (mg/l)
Fill and draw	1,000	1,150	6,000	6,200	No data	1,700
Batch	415	315	6,000	6,200	2,000	1,700

entirely favors bacterial biomass because the system operates on the portion of the respirometric curve where yeast are not producing new cells. When the research group ran CSTRs, no matter how conditions were adjusted to favor yeast, bacteria came to dominate the system over time. This same result was found Zheng et al. [7] when they ran a continuous feed system.

This does not necessarily mean that yeast systems cannot be designed to treat low COD wastewater. Low COD respirometric experiments (COD < 500) resulted in low COD effluent. However, the biomass must be very thin and loading on that biomass must still be high. A plug flow type system is one option for a continuous feed yeast system that would meet the instantaneous loading requirements.

4.6.2. Remove biomass from effluent after primary substrate has been degraded

Lowest COD's in the reactors were achieved immediately after all substrate was consumed. The longer the reactors were allowed to sit after substrate was consumed, the larger the effluent COD became due to the large amount of SMP that is produced by yeast consuming stored material. In order to achieve best water treatment, yeast should be separated from the effluent as soon as possible after primary substrate has been completely consumed.

4.6.3. Mass of sludge and sludge handling can be greatly reduced by a few days of digestion

Stored substrate was found to have a direct 1:1 relationship to MLSS. This stored carbohydrate, when consumed, becomes SMP and CO_2. This means that MLSS is directly reduced as the stored material is consumed. After 3 d, yeast sludge mass can be reduced by 44% or more.

In bacterial systems, digestion of sludge reduces biomass. The biomass degrades at a rate of 0.2 1/d. Therefore, after 3 d, 49% of the active biomass should be degraded. However, this degraded material leaves a large amount of non-biodegradable solids. So, while the active biomass dies and is reduced, the MLSS does not have an equivalent reduction in mass like in the yeast reactor.

4.6.4. Secondary treatment must be capable of destroying/consuming SMP

The effluent from yeast reactors contains SMP that is not easily degraded. In order to polish this wastewater, either an organism/sludge must be found that can efficiently consume the material or it must be oxidized to reduce COD.

Ozonation of the SMP is an effective method for reduction of SMP that the research group has used on high SMP effluent. Although it has not yet been tried on yeast sludge effluent, ozonation followed by a small biological system likely can remove most of the SMP remaining after treatment of the high strength wastewater.

5. Conclusion

The research presented here has shown that aerobic yeast metabolism in the absence of fermentation, while similar in some ways to that of bacteria, has many unique characteristics. These differences are manifested when concentration of carbon source becomes limited in the bulk liquid. At that time, yeast cell production stops and storage/conversion of carbon source to carbohydrate begins. Yeast consume these carbohydrate reserves when carbon source has been depleted.

ASM modeling is unable to produce curves that coincide with yeast respirograms. YASM uses processes and mathematical computations that are recognizable to scientists familiar with ASM. However, YASM is capable of modeling the kinetics observed in yeast respirograms.

As a result of YASM, it is now possible to understand how best to operate YAS systems and why conventional continuous flow yeast systems are untenable. Recovery of yeast systems that have been contaminated by bacterial biomass can be easily accomplished when the model is understood. System recovery is accomplished by modifying operation to maintain exponential growth conditions.

Yeast have great potential as a wastewater treatment organism, especially for the degradation of waste streams at high salinity, low pH waste streams, and waste streams containing particular organic constituents that are well suited for yeast degradation. YASM can be an important tool moving forward for studying, evaluating, and designing YAS systems.

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