

Optimal Operating Points for SCP Production in the U-Loop Reactor

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Abstract: The microorganism *Methylococcus capsulatus* can grow on cheap carbon sources such as methane and methanol. *M. capsulatus* has a protein content of approximately 70% and can be used for production of so-called Single Cell Protein (SCP). Static simulations of SCP production using *Methylococcus capsulatus* in an U-loop reactor have been used to determine the optimal operation. The optimal operating point is located close to both washout and oxygen limitation. With oxygen being the most expensive reactant, the U-Loop reactor is operated in the oxygen limited mode and substrate feed is controlled according to the oxygen feed. The maximum oxygen feed is determined by the maximum biomass concentration that can be tolerated in the reactor. Higher biomass concentration is believed to give a more viscous fluid that requires more pumping energy for circulation in the U-loop and has a lower gas-liquid oxygen transfer rate. The optimal dilution rate is relative constant around 0.2 1/hr. This operating strategy gives the highest SCP productivity.

Keywords: U-Loop Reactor, Single Cell Protein, Modeling and Simulation, Fermentation

1. INTRODUCTION

With the increasing world population, the demand for proteins increases. Proteins and amino acids are essential for growth of humans and animals. Proteins cannot be substituted by other food components. So far, soya beans and fish in the form of fish meal have been important sources of protein for animal feed. In turn, the animals have served as protein sources in human nutrition. However, with the diminishing fish reserves in the ocean, it will be a challenge to sustain the protein demand of an increasing population. Technological advancements must provide new ways to synthesize proteins and produce them in a cost efficient manner.

The microorganism *Methylococcus capsulatus* can grow on cheap carbon sources such as methane and methanol (Papoutsakis et al., 1978; Hanson and Hanson, 1996; Bothe et al., 2002). The protein content of *M. capsulatus* is approximately 70% on a dry mass basis. This protein is called Single Cell Protein (SCP) as it is produced in single cells. In addition to methanol, the process requires oxygen and sources for nitrogen and minerals. We use nitric acid as the nitrogen source. Consequently, proteins for animal feed may be produced by fermentation of *M. capsulatus* using methanol, oxygen, nitric acids, and minerals as the feed stocks. This is illustrated in Figure 1. The cells are hydrolyzed to improve digestibility of the proteins (Villadsen, 2002).

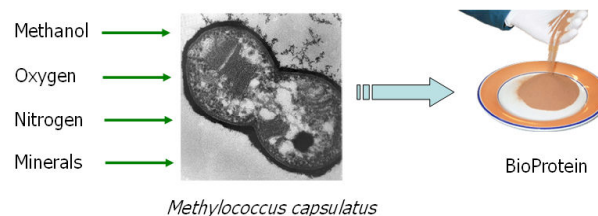


Fig. 1. Production of bio protein (single cell protein) using *Methylococcus capsulatus*.

Unlike many other bio-chemicals, Single Cell Protein is a commodity. Therefore, it is essential that the process equipment for manufacturing of Single Cell Protein is energy efficient and able to utilize the raw materials with a high yield. The principle problems facing the manufacture of Single Cell Protein is transfer of oxygen to the liquid phase and removal of the high amount of heat produced by the exothermic process. Conventional stirred tank fermenters cannot provide sufficient mass transfer of oxygen nor sufficient area for removal of the heat produced. The U-loop reactor is a reactor designed to have high degrees of gas-liquid mixing and heat removal. The U-loop reactor at the Technical University of Denmark is illustrated in Figure 2. The legs (the u-loop) of the reactor are equipped with static mixers to have high gas-liquid mass transfer rates. The u-loop is also equipped with a heat exchanger for removal of the extensive heat produced by the fermentation. The tank on the top of the legs is essentially a degassing unit. It is used to separate the

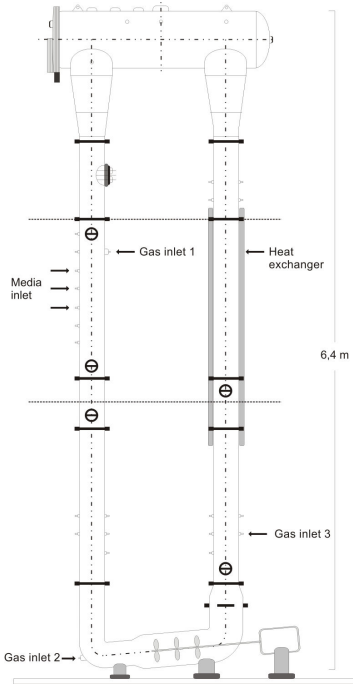


Fig. 2. The U-loop reactor pilot plant at the Technical University of Denmark.

produced CO_2 from the liquid. Furthermore, as long as the substrates (methanol, oxygen, nitric acid, and minerals) are present, the micro organism will also grow in the tank at the top. The U-Loop reactor at DTU is used for production of SCP based on methanol, oxygen, nitric acid (potassium nitrate), and minerals.

Few references are available on the modeling, control and optimization of SCP production. Scrimshaw et al. (1968) provides a comprehensive description of SCP production. Alvarez and Ricano (1979) modeled and optimally controlled yeast grown on methanol for SCP production in a batch reactor. The SCP producing U-loop reactor has been modeled to facilitate design of pH and temperature controllers along with synthesis of an optimal start-up procedure (Prado Rubio, 2007; Prado Rubio et al., 2009). A model intended for receding horizon optimizing control of SCP production in the U-loop reactor has also been developed (Olsen, 2009; Olsen et al., 2009). Bailey and Ollis (1986) as well as Nielsen et al. (2003) provide general introductions to fermentation technology.

In this paper we use the model developed by Olsen et al. (2009) to determine optimal static operation points for SCP production in the U-loop reactor. The model consisting of stoichiometry, kinetics, and conservation equations is summarized in Sections 2 and 3. Section 4 provides results from static simulation and optimization using the model. Finally, Section 5 discuss and concludes on the results.

2. STOICHIOMETRY AND KINETICS FOR GROWTH OF *M. CAPSULATUS*

In this section we describe the stoichiometry and kinetics related to growth of *M. capsulatus*.

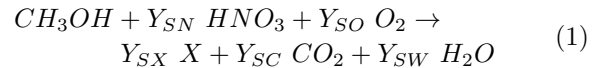
Table 1. Yield coefficients

	i	Y_{Si} [mol/mol]	Y_{Xi} [mol/mol]	M_{wi} [g/mol]	W_{Xi} [g/g]
CH_3OH	S	1.000	1.366	43.773	1.778
HNO_3	N	0.146	0.199	63.013	0.510
O_2	O	0.439	0.600	31.999	0.779
$\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2}$	X	0.732	1.000	24.626	1.000
CO_2	C	0.268	0.366	44.010	0.654
H_2O	W	1.415	1.933	18.015	1.414

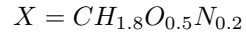
Table 2. Kinetic parameters

Symbol	Value	Unit
μ_{\max}	0.37	1/hr
K_S	0.021	kg/m ³
K_I	0.38	kg/m ³
K_O	$6.4 \cdot 10^{-5}$	kg/m ³

Growth of *M. capsulatus* from methanol can be approximated by the overall reaction



in which X symbolizes the biomass having the molecular formula



The yield coefficients, Y_{ji} , for reactions (1) are presented in Table 1.

The specific growth rate of *M. capsulatus* from methanol and oxygen, μ , can be described by the Monod-Haldane expression

$$\mu = \mu(C_S, C_O) = \mu_{\max} \cdot \mu_S(C_S) \cdot \mu_O(C_O) \quad (2a)$$

in which

$$\mu_S(C_S) = \frac{C_S}{K_S + C_S + (C_S/K_I)^2} \quad (2b)$$

$$\mu_O(C_O) = \frac{C_O}{K_O + C_O} \quad (2c)$$

C_S is the substrate (CH_3OH) concentration (kg/m³) and C_O is the concentration of dissolved oxygen (kg/m³). The parameters of these expressions at $T = 45^\circ\text{C}$ and $pH = 7.0$ are provided in Table 2. The specific growth rate, μ , may be used to compute the production rate of biomass

$$R_X = \mu C_X \quad (3)$$

C_X is the concentration of biomass (kg/m³) and R_X is the production rate of biomass (kg/m³/hr).

The production rates (consumption rates) of methanol (S) and dissolved oxygen (O) may be computed based on the stoichiometry and the production rate of biomass

$$R_S = -\frac{M_{wS}}{M_{wX}Y_{SX}}R_X = -\gamma_S R_X \quad (4a)$$

$$R_O = -\frac{M_{wO}Y_{SO}}{M_{wX}Y_{SX}}R_X = -\gamma_O R_X \quad (4b)$$

R_S is the production rate (consumption rate) of methanol (kg/m³/hr) and R_O is the production rate (consumption rate) of dissolved oxygen (kg/m³/hr).

3. U-LOOP REACTOR MODEL

The U-loop reactor (Figure 2) is modeled as illustrated in Figure 3. The model consists of a dynamic continuous

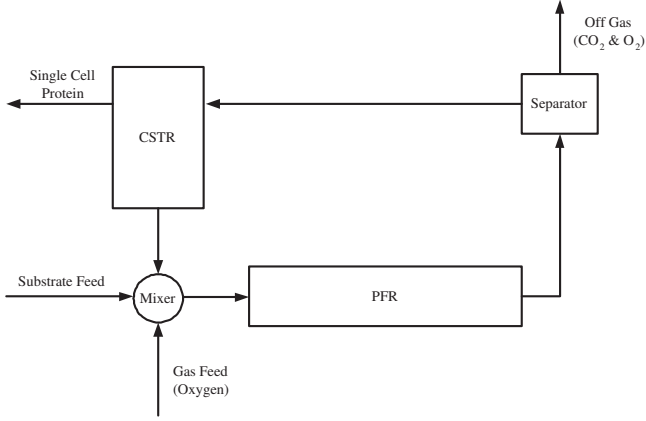


Fig. 3. Model of the U-Loop reactor.

stirred tank reactor (CSTR), a static mixer, a dynamic plug flow reactor (PFR), and a static ideal gas-liquid separator. The static models are trivial and we only present the dynamic CSTR and PFR models.

3.1 CSTR Model

The CSTR model constitute the liquid phase of the top tank in the U-Loop Reactor. Since there is very little gas-liquid interface no oxygen is transferred from the gas to the liquid phase or vice versa. It is therefore sufficient to model the liquid phase.

The evolution of the liquid phase concentrations (kg/m^3) can be described as

$$\frac{dC_X}{dt} = DC_{X,in} - DC_X + R_X \quad (5a)$$

$$\frac{dC_S}{dt} = DC_{S,in} - DC_S + R_S \quad (5b)$$

$$\frac{dC_O}{dt} = DC_{O,in} - DC_O + R_O \quad (5c)$$

in which the dilution factor is given as

$$D = \frac{F}{V} \quad (6)$$

with F being the total flow rate (m^3/hr) in and out of the reactor and V being the volume of the liquid phase (m^3).

3.2 PFR Model

The PFR model contains a gas phase and a liquid phase. Let ϵ describe the volumetric gas phase fraction

$$\epsilon = \frac{F_G}{F_L + F_G} \quad (7)$$

in which F_G is the volumetric gas phase flow rate (m^3/hr) and F_L is the volumetric liquid flow rate (m^3/hr). The linear velocity, v (m/hr), in the reactor is

$$v = \frac{F_L + F_G}{A} \quad (8)$$

in which A (m^2) is the cross-sectional area of the pipes constituting the u-loop.

The evolution of the concentrations in the liquid phase may be described by

Table 3. Transport Parameters

Symbol	Value	Unit
$(k_l a)_O$	120	1/hr
H_O	24.707	(atm · m ³)/kg
D_X	103.68	m ² /hr
D_S	103.68	m ² /hr
D_O	103.68	m ² /hr
D_{gO}	103.68	m ² /hr

$$\frac{\partial C_X}{\partial t} = -\frac{\partial N_X}{\partial z} + R_X \quad (9a)$$

$$\frac{\partial C_S}{\partial t} = -\frac{\partial N_S}{\partial z} + R_S \quad (9b)$$

$$\frac{\partial C_O}{\partial t} = -\frac{\partial N_O}{\partial z} + R_O + \frac{1}{1-\epsilon} J_{gl,O} \quad (9c)$$

in which N_i ($\text{kg}/\text{m}^2/\text{hr}$) is the mass flux of component i , R_i ($\text{kg}/\text{m}^3/\text{hr}$) is the production rate of component i , and $J_{gl,O}$ ($\text{kg}/\text{m}^3/\text{hr}$) is the mass transfer rate of oxygen from the gas phase to the liquid phase.

A mass balance for oxygen in the gas phase (gO) provides the partial differential equation

$$\frac{\partial C_{gO}}{\partial t} = -\frac{\partial N_{gO}}{\partial z} - \frac{1}{\epsilon} J_{gl,O} \quad (10)$$

We assume that no reaction occurs in the gas phase.

The flow in the reactor is assumed to be a combination of convective flow, vC_i , and dispersive flow, J_i ,

$$N_i = vC_i + J_i \quad i \in \{X, S, O, gO\} \quad (11)$$

with the dispersive flow governed by Fick's law

$$J_i = -D_i \frac{\partial C_i}{\partial z} \quad i \in \{X, S, O, gO\} \quad (12)$$

The mass transfer of oxygen from the gas phase to the liquid phase is described by the linear relation

$$J_{gl,O} = (k_l a)_O (C_{O,sat} - C_O) \quad (13)$$

in which the saturation concentration is computed using Henry's law

$$C_{O,sat} = \frac{P_O}{H_O} = \frac{RT}{M_{wO} H_O} C_{gO} \quad (14)$$

The transport parameters for the U-Loop Reactor has been determined experimentally and are provided in Table 3 (Andersen et al., 2005a,b; Soland, 2005; Piper, 2004).

4. STATIC SIMULATION AND OPTIMIZATION

The model describing fermentation of *M capsulatus* in the U-loop reactor may by finite-volume spatial discretization be converted to a system of ordinary differential equations (Olsen et al., 2009)

$$\dot{x}(t) = f(x(t), u) \quad x(t_0) = x_0 \quad (15)$$

with x being the states in the model and u being the manipulable variables (MVs) of the model. We consider the recirculation flow rate (flow rate to mixer from CSTR), F_R , to be fixed at its upper bound. Therefore, the MVs in the model are the feed and product flow rate, F [m^3/hr], the gas feed flow rate, F_G [m^3/hr], and the substrate (methanol) flow rate, m_S [kg/hr]. $u = [F \ F_G \ m_S]'$. The concentration of substrate in the feed is $C_{F,S} = m_S/F$.

The steady states as function of the MVs, $x_s = x_s(u)$, is determined by numerical solution of the system of nonlinear equations

Table 4. Parameters for the U-loop reactor.

	Symbol	Value	Unit
Gas flow rate	F_G	0.3240	m ³ /hr
Oxygen fraction in gas feed	y_{O_2}	0.97	
Recycle flow rate	F_R	79.65	m ³ /hr
Linear velocity of fluid in PFR	v_z	1	m/s
Gas fraction in PFR	ϵ	0.0041	
CSTR volume	V_{CSTR}	0.250	m ³
U-Loop Length	L	12.0	m
Cross sectional area	A_{PFR}	0.0222	m ²
Pressure	P	5	atm
Temperature	T	314	K
No. of PFR Control Volumes	N_d	20	

$$f(x_s, u) = 0 \quad (16)$$

These states are used to determine the optimal operating point, u^* . We use the productivity per unit volume U-loop reactor to select the optimal operating point. Define the dilution rate of the U-loop reactor as

$$D_{ULoop} = \frac{F}{V_{ULoop}} = \frac{F}{V_{CSTR} + A_{PFR}L} \quad (17)$$

where F [m³/hr] is the flow rate of the liquid feed and the SCP product stream. Then the productivity per unit reactor volume is

$$P_X = D_{ULoop} \cdot C_X \quad (18)$$

with C_X [kg/m³] being the concentration of biomass in the CSTR (the degassing tank in the top of the U-loop reactor).

The static simulations in the following subsections are made using the parameters in Table 4.

4.1 Operation Window with Fixed Gas Flow Rate

We consider the static behavior for fermentation of *M. capsulatus* in the U-loop fermenter for a fixed gas flow rate ($F_G = 0.3240$ m³/hr) and varying values of the product flow rate, F , and the substrate feed rate, m_S . The product flow rate, F , is specified in terms of the dilution rate, D_{ULoop} . We consider all combinations of dilution rate and substrate flow rate in the range

$$D_{ULoop} \in [0.0697 \ 0.2231] \frac{1}{h}$$

$$m_S \in [1.8000 \ 4.6033] \frac{kg}{h}$$

and compute steady states by solution of (16) for all combinations of D_{ULoop} and m_S in the specified range.

Figure 4 illustrates the productivity, the gas phase oxygen concentration (C_{gO}) at the outlet of the PFR, and the biomass concentration (C_X) in the CSTR as function of the dilution rate (D_{ULoop}) and the substrate feed rate (m_S).

Washout and zero productivity occurs if D_{ULoop} is higher than approximately 0.2 1/hr. The limit is governed by the specific growth rate, μ , in the U-loop fermenter.

Similarly, washout and zero productivity occurs if the substrate feed, m_S , is higher than approximately 4.0 kg/hr. In this case, we feed more methanol than consumed by the growth of *M. capsulatus* and removed in the product stream. Methanol accumulates in the reactor and high methanol concentrations inhibits the growth

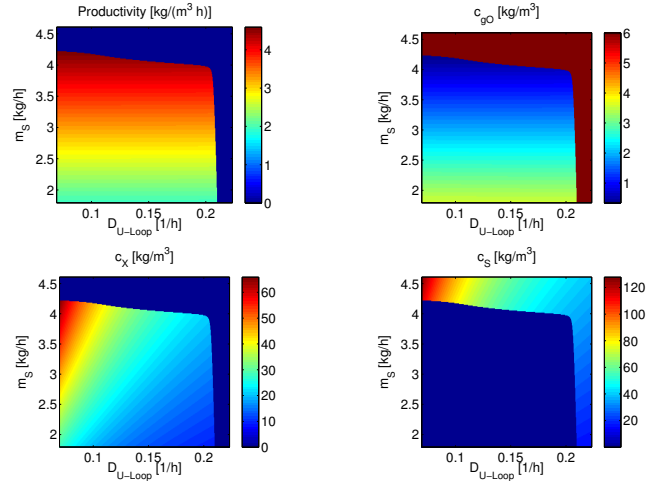


Fig. 4. Productivity, outlet oxygen concentration, biomass concentration (CSTR), and substrate concentration (CSTR) in the U-loop reactor as function of m_S and D_{ULoop} for $F_G = 0.3240$ m³/hr.

of *M. capsulatus* until the point at which growth stops completely. Consequently, all biomass is washed out of the reactor no matter how small the dilution rate. This upper bound in the substrate feed rate is limited by the oxygen feed and transfer rate as illustrated by the C_{gO} -plot in Figure 4. Washout occurs when there is no more oxygen in the gas phase at the outlet of the PFR. This implies that we have fed an excess of substrate compared to the oxygen feed and substrate accumulates in the reactor.

At a fixed gas flow rate, the optimal productivity is achieved when oxygen is the limiting reactant, i.e. at a substrate feed rate of approximately 4.0 kg/hr in this case (see Figure 4). At a fixed substrate feed rate, the highest productivity comes at low dilution rates. However, this is accompanied with high biomass concentration. The fluid becomes more viscous as the biomass concentration increases. This increases the pumping costs associated with circulating the medium in the legs of the U-loop reactor and also decreases the specific mass transfer rate of oxygen. The latter effect is not included in the model though. Both the consideration of high productivity and low biomass concentration must be taken into account when deciding on the optimal operating point at a given gas flow rate. With these considerations in mind, the optimal operating point is $(D_{ULoop}^*, m_S^*) \approx (0.2 \text{ 1/hr}, 4.0 \text{ kg/hr})$. This point is located in the upper right corner of the productive operating window.

To have a simplified operating strategy when we vary the gas feed flow rate, we back-off from the oxygen limit and choose $(D_{ULoop}, m_S) = (0.2 \text{ 1/hr}, 3.853 \text{ kg/hr})$ as the operating point for a gas feed flow rate of $F_G = 0.3240$ m³/hr. Figure 5 illustrates the static concentration profiles at this operating point. Figure 6 illustrates the associated specific growth rate, μ , and the oxygen transfer rate, $J_{gl,O}$. Notice that the specific growth rate μ is approximately equal to the maximal dilution rate.

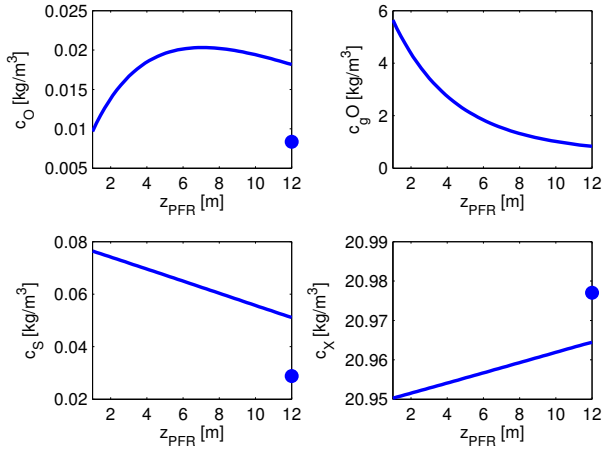


Fig. 5. Concentration profiles in the loop reactor for $(D_{U_{Loop}}, m_S, F_G) = (0.2 \text{ 1/hr}, 3.853 \text{ kg/hr}, 0.3240 \text{ m}^3/\text{hr})$. The dot denotes the concentration in the CSTR.

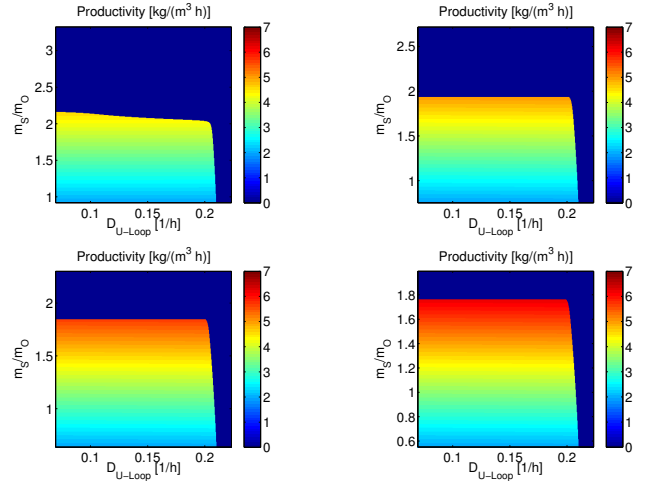


Fig. 7. Productivity, P_X , as function of dilution rate, $D_{U_{Loop}}$ and substrate-oxygen ratio, m_S/m_O for various gas flow rates, F_G (A: upper left, B: upper right, C: lower left, D: lower right).

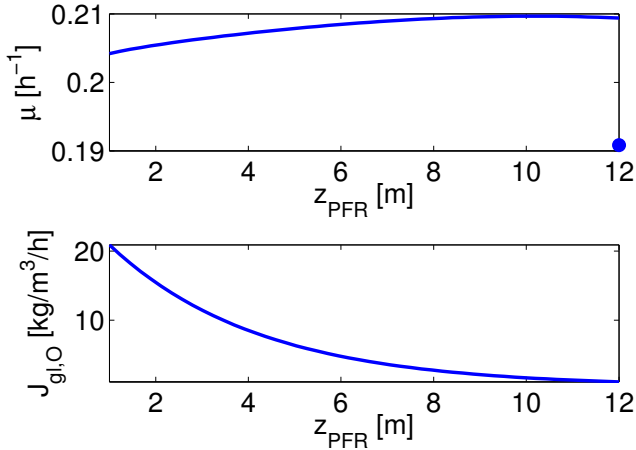


Fig. 6. Specific growth rate and oxygen mass transfer for $(D_{U_{Loop}}, m_S, F_G) = (0.2 \text{ 1/hr}, 3.853 \text{ kg/hr}, 0.3240 \text{ m}^3/\text{hr})$. The dot denotes the specific growth rate in the CSTR.

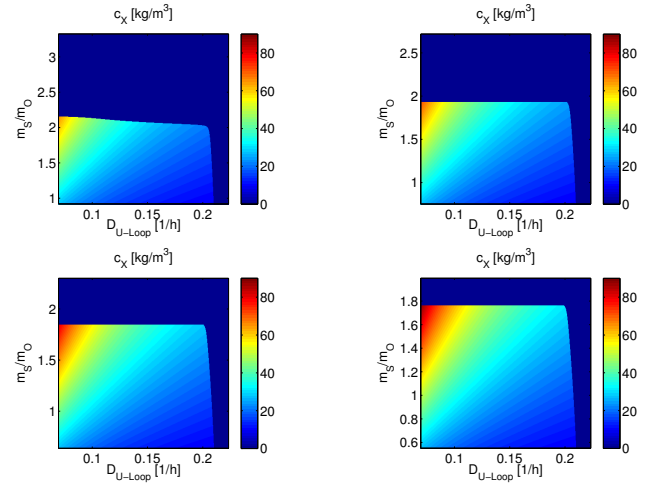


Fig. 8. Biomass concentration, C_X , as function of dilution rate, $D_{U_{Loop}}$ and substrate-oxygen ratio, m_S/m_O for various gas flow rates, F_G (A: upper left, B: upper right, C: lower left, D: lower right).

Table 5. Various gas flow rate operating points

Operating Point	F_G m ³ /hr	ϵ	m_O kg/hr	m_S/m_O	$D_{U_{Loop}}$ 1/hr
A	0.3240	0.0041	1.9516	1.7157	0.2
B	0.3960	0.0050	2.3852	1.7136	0.2
C	0.4680	0.0058	2.8190	1.7121	0.2
D	0.5400	0.0067	3.2526	1.7118	0.2

4.2 Operation Window with Varying Gas Flow Rate

The effect of gas feed flow rate, F_G , on the feasible operating range of the dilution rate, $D_{U_{Loop}}$, and the oxygen flow rate, m_O , as well as the effect on the productivity, P_X , is investigated. We repeat the computations and analysis of the operating window performed for a fixed gas flow rate (Section 4.1) at 4 different gas flow rates. The chosen gas flow rates are given in Table 5.

The ratio of methanol to oxygen, m_S/m_O , that needs to be fed to the reactor is determined by the reaction stoichiometry (1), and losses of methanol and oxygen in the effluent streams. Without losses in the effluent streams, reaction stoichiometry dictates a constant ratio $m_S/m_O = 2.2824 \text{ kg CH}_3\text{OH} / \text{kg O}_2$. However, losses of methanol and oxygen in the effluent streams change this ratio and may make it dependent on the operating point. The plots in Figure 7 and Figure 8 illustrate the productivity and biomass concentration as function of the dilution rate, $D_{U_{Loop}}$, and the feed methanol-oxygen ratio, m_S/m_O , at the four gas flow rates, F_G , listed in Table 5. At all gas flow rates, the maximal dilution rate without washout is $D_{U_{Loop}} \approx 0.2 \text{ 1/hr}$. Maximal productivity is obtained when all oxygen is consumed and it becomes the limiting reactant. The optimal methanol-oxygen ratio, m_S/m_O , is only weakly dependent on the dilution rate, $D_{U_{Loop}}$, but depends on the gas feed flow rate, F_G (or m_O). The optimal methanol-oxygen ratio, m_S/m_O , decreases with

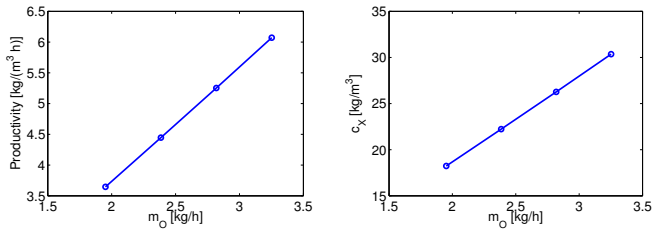


Fig. 9. Productivity, P_X , and biomass concentration, C_X , as function of oxygen gas flow rate, m_O , for the operating points in Table 5.

increasing gas flow rate. This implies that the oxygen utilization decreases with increasing gas flow rate.

As all ready noted, the maximal productivity at all gas flow rates is obtained when oxygen becomes a limiting reactant. At a fixed methanol-oxygen ratio, m_S/m_O , the productivity is independent of the dilution rate, D_{ULoop} , in the productive dilution rate range. At a fixed methanol-oxygen ratio, m_S/m_O , the biomass concentration increases with decreasing dilution rate. The optimal ratio of methanol to oxygen, m_S^*/m_O , is almost independent of the dilution rate, D_{ULoop} . Consequently, at any fixed gas flow rate, F_G (or m_O), the maximum productivity at lowest biomass concentration is achieved in the upper right corner of the productive operating window. This can be observed in Figure 7 and Figure 8. Due to increased loss of oxygen at high gas flow rates, the optimal methanol-oxygen ratio, m_S/m_O , decreases with increasing gas flow rate.

Next we choose the operating point, $(D_{ULoop}, m_S/m_O) \approx (0.2 \text{ 1/hr}, 0.75 W_{OS}) = (0.2 \text{ 1/hr}, 1.71)$. $W_{OS} = W_{XS}/W_{XO} = 2.2824$ is the stoichiometric methanol-oxygen ratio. This operating point rule gives a feasible but suboptimal operating strategy for the range of gas flow rates considered. The operating points in Table 5 are constructed using this strategy. Figure 9 shows the productivity, P_X , and biomass concentration, C_X , for the operating points in Table 5 as function of the oxygen feed rate, m_O . A higher productivity can be obtained by increasing the gas flow rate. This higher productivity comes at the expense of higher biomass concentration and lower oxygen utilization. The higher biomass concentration implies higher fluid viscosity and pumping costs.

Consequently, the highest biomass concentration that can be tolerated gives the maximum oxygen feed rate, m_O . This oxygen feed rate, m_O , determines the substrate feed rate, m_S , and the productivity, P_X . The optimal dilution rate is constant at $D_{ULoop} \approx 0.2 \text{ 1/hr}$.

5. CONCLUSION

Static simulations of Single Cell Protein (SCP) production using *Methylococcus capsulatus* in an U-loop reactor have been used to determine the optimal operation. The optimal operating point is located close to both washout and oxygen limitation. With oxygen being the most expensive reactant, the U-Loop reactor is operated in the oxygen limited mode and substrate feed is controlled according to the oxygen feed. The maximum oxygen feed is determined by the maximum biomass concentration that can

be tolerated in the reactor. Higher biomass concentration is believed to give a more viscous fluid that requires more pumping energy for circulation in the U-loop and has a lower gas-liquid oxygen transfer rate. The optimal dilution rate is relative constant around 0.2 1/hr. This operating strategy gives the highest SCP productivity.

Future studies should investigate the optimal dependence of substrate feed rate to oxygen feed rate, as well as the dynamic consequences of choosing the suggested operating strategy.

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