

Full Paper

DESIGN OF A CONTINUOUS BIOREACTOR FOR LABORATORY SCALE PRODUCTION OF ETHANOL FROM CASSAVA STARCH HYDROLYSATE USING SACCHAROMYCES CEREVISIAE

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ABSTRACT

In this work, Microsoft excel spreadsheet was employed to design a 5L laboratory scale continuous bioreactor with kinetic data obtained from the previous batch fermentation process for bioethanol production from cassava starch hydrolysate using *Saccharomyces cerevisiae*. This was achieved by dividing the whole system into various modules (Vessel dimensions, Agitation system design, and Power requirement for agitation, Material and Energy Balance, and / or Temperature control) and linking various cells from the spreadsheet for each module with one and other. The vessel height and diameter are 0.30 m and 0.15 m, respectively using the aspect ratio of 2. The agitation system comprises 4 baffles and 3 six-blade rushton turbine impellers (speed of 200 rpm and power required for agitation calculated as 5.2×10^{-4} W). The mass flow rates of feed and of ethanol in the product stream are 9.11×10^{-6} kg/s and 2.2×10^{-6} kg/s, respectively. The system is to be maintained at 30 °C using tap-water as coolant. It also has 4.88×10^{-4} m² as the area available for heat transfer and the length of the cooling coil required for this is calculated as 6.63×10^{-3} m.

Keywords: Bioethanol production, Bioreactor design, Cassava starch, *Saccharomyces cerevisiae*.

Nomenclature

Symbol	Nomenclature	Unit
Ac	Area available for cooling	m ²
Cpw	Specific heat capacity of cooling water	KJKg ⁻¹ K ⁻¹
D _T	Diameter of the vessel	m ²
Dopt	Optimal dilution rate	h ⁻¹
F	flow rate	m ³ h ⁻¹
Ff	fouling factor	KWm ⁻² K ⁻¹

Fw	volumetric flow rate of feed	m ³ h ⁻¹
Hrxn	heat of reaction	KJ
H _T	height of the vessel	m
Ks	limiting substrate concentration	gL ⁻¹
Kcoil	thermal conductivity of cooling coil	KWm ⁻¹ K ⁻¹
Kcw	thermal conductivity of cooling water	KWm ⁻¹ K ⁻¹
Lc	length of the cooling coil	m
Mfw	mass flow rate of cooling water	Kgh ⁻¹
Mw	mass flowrate of feed	Kgh ⁻¹
Ni	Agitation speed	s ⁻¹
Np	Power number	
Pt	maximum concentration of ethanol	gL ⁻¹
Pw	power required for agitation	W
Qacc	rate of heat accumulation in the system	KJ/s
Qexch	heat loss by the system	KJ/s
Qeva	heat loss by the system	KJ/s
Qgas	heat loss by the system	KJ/s
Qsen	heat loss by the system	KJ/s
Q	Specific productivity	gg ⁻¹ h ⁻¹
Qp	volumetric productivity	gL ⁻¹ h ⁻¹
Qs	volumetric rate of substrate consumption	gL ⁻¹ h ⁻¹
Rei	Reynoldid number	
R	radius of the coil	m
S	initial substrate concentration	gL ⁻¹
Si	final substrate concentration	gL ⁻¹
Trgt	Targeted temperature	0°C
Tm	mean temperature	0°C
Tenti	Cooling water temperature (in)	0°C
Tento	Cooling water temperature (out)	0°C
U	overall heat transfer coefficient	KWm ⁻² K ⁻¹
V _T	volume of the vessel	m ³
Vwr	percentage working volume of the vessel	
Vw	volumetric flowrate of cooling water	m ³ /s
Ws	shaft work	KJ
x	cooling coil thickness	m
Yxs	biomass yield	gg ⁻¹
Yps	process product yield from substrate	gg ⁻¹
Ypx	specific product yield	gg ⁻¹
Yco ₂	CO ₂ yield	gg ⁻¹
Yetoh	percentage theoretical yield	
ρ	density of fermentation broth	kgm ⁻³
μ	specific growth rate	h ⁻¹
μmax	maximum specific growth rate	h ⁻¹

1. INTRODUCTION

The efforts to find sustainable alternatives to the fossil fuel continue to receive increasing interest. Bioethanol had been proven one of the best alternatives to the conventional fossil fuel (Bai et al., 2008). Nigeria in her recent efforts to commercialize bioethanol production has chosen cassava as one of the biomaterials due to its significant abundance. In Nigeria, there are various research efforts such as Betiku et al. (2010) on ethanol production from cassava.

Although Anozie et al. (2005) evaluated a batch bio-digester for biogas production from agricultural wastes; such efforts in the area of bioreactor design for bioethanol production have rarely been reported. Bioreactors are the apparatus in which practical biochemical reactions (such as bioethanol fermentation) are performed (Katoh and Yoshida, 2009). A typical example of bioreactor is shown in the Figure 1.

Apart from the hand calculation and simulation software such as hyprotech HYSYS, Excel MS spreadsheet has been used in bioreactor design (Gimbun et al., 2004). The use of excel eliminates the need for employing expensive simulation software and labouring over hand calculations. Besides, it is easy to use and allows for possible scale up and process simulation (Gimbun et al., 2004). In this work, Microsoft excel spreadsheet is employed to design a 5L laboratory scale continuous bioreactor using the kinetic data obtained from the batch fermentation process for bioethanol production from cassava starch hydrolysate (Alade, 2010). This was achieved by dividing the whole system into various modules (Vessel dimensions, Agitation system design, and Power requirement for agitation, Material and Energy Balance, and/or Temperature control) and linking various cells from the spreadsheet for each module with one and other.

It has been stated that an order of magnitude of the proposed chemical plant is always determined through preliminary design calculation of a unit operation (Gimbun et al., 2004). Hence, laboratory scale fermentor would provide basic information for the pilot plant design and subsequently the industrial scale design. Moreover, for a nation (such as Nigeria) which is critically in need of technological advancement to drive its economy and find alternative energy sources, the design of bioreactor for bioethanol production is necessary.

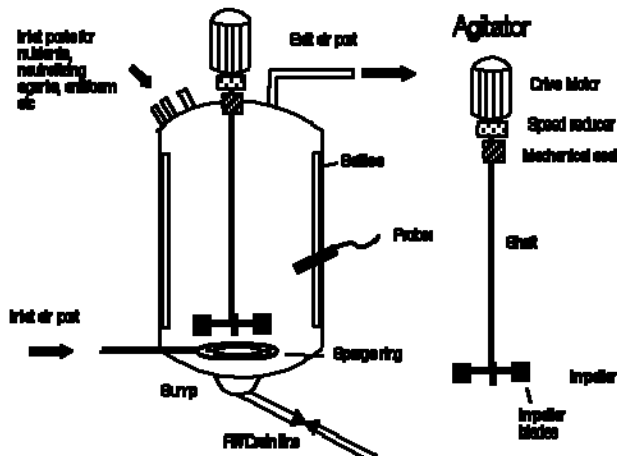


Fig. 1: A representation of bioreactor

2. METHODOLOGY

2.1 Batch fermentation process and kinetic data

Three different fermentation runs were carried out in a 7-liter bench scale bioreactor (Alade, 2010). Complex medium containing (per liter) 100 g reducing sugar equivalent of cassava starch hydrolysate (CSH), 5 g of yeast extract, 2 g KH_2PO_4 , 1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and minimal medium containing (per liter) 100 and 120 g/L glucose equivalent of the (CSH), 2 g $(\text{NH}_4)_2\text{SO}_4$, 2 g KH_2PO_4 , and 1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were employed for the studies. The fermentation media were inoculated with 5 percent fully grown inoculum of *Saccharomyces cerevisiae* and were cultivated at temperature of 30 °C and pH of 4.5. Samples were collected at

regular intervals and were analyzed for ethanol reducing sugar and biomass for 24 h.

The batch kinetic parameters were calculated as described by (Lawford and Rousseau, 1993) and (Rajoka et al., 2005). The volumetric productivity of ethanol, Q_p ($\text{g L}^{-1} \text{h}^{-1}$) was determined from a plot between ethanol concentration (g L^{-1}) and fermentation time (h). Also, volumetric rate of substrate consumption, Q_s ($\text{g L}^{-1} \text{h}^{-1}$) was determined from a plot between reducing sugar concentration (g L^{-1}) and fermentation time (h). Process product yield (Y'_{ps}) was determined from dPt/dS . Specific product yield (Y'_{px} , g ethanol g^{-1} cells) was determined using the relationship dPt/dX . The values of dPt/dS and dPt/dX were both obtained as described by (Layokun and Solomon, 1989) and (Doran, 1995). The plot of ethanol concentration versus substrate (reducing sugar) concentration and manually drawing tangents gives dPt/dS . Similarly, the plot of ethanol concentration versus biomass concentration and manually drawing tangents gives dPt/dX . Specific productivity, Q ($\text{g g}^{-1} \text{h}^{-1}$), was determined multiplying Y'_{px} by μ (specific growth rate). Specific growth rate (μ) was determined as slope of a straight line between $\ln X/X_0$ (X is the biomass, g L^{-1} and X_0 is the biomass at the start of exponential growth phase) and fermentation time (h). Growth yield coefficient (Y'_{xs}) that is, g cells formed per g substrate utilized was obtained by plotting X (g/L) cells versus substrate concentration (g/L).

2.2 Bioreactor geometry

Stirred (agitated) tanks, which are widely used as bioreactors (especially as fermenters), are vertical cylindrical vessels equipped with a mechanical stirrer (agitator) or stirrers that rotate around the axis of the tank (Katoh and Yoshida, 2009). The geometrical ratios of such vessels have been severally presented in the literatures (Bailey and Ollis, 1986; McCabe et al., 1993; Stanbury et al., 2000; Doran, 2005; Katoh and Yoshida, 2009). Typical bioreactor geometrical ratio is presented in the excel spreadsheet in Table 1.

2.2.1 The vessel dimensions

The vessel capacity is 5 L ($V_T = 0.005 \text{ m}^3$) and the other parts are related to the tank diameter D_T as shown in the excel page (Table 1).

The volume of the cylindrical vessel is determined with eqn (1):

$$V_T = JI (D_T^2/4) H_T \quad (1)$$

With the aspect ratio of 2.0, $V_T = JI (D_T^3/2)$ and $D_T = 0.15 \text{ m}$. Using equation 1, the other dimensions were calculated as shown in Table 1.

2.3 Agitation system design

In the continuous stirred tank reactor (CSTR), the reactor contents are perfectly mixed and uniform through out (Katoh and Yoshida, 2009). The agitation system comprises the shaft, baffle and impeller, which also comprise the impeller disc and blade. Mixing is achieved using an impeller mounted in the tank. It is usually positioned overhead on a centrally locate stirrer shaft. The most frequently used impeller in the fermentation industry is the 6 flat-blade disc mounted turbine – Rushton turbine (Doran, 1995). The standard dimensions of rushton turbine relative to the tank size are available in the literature (McCabe et al., 1993; Doran, 2005; Katoh and Yoshida, 2009).



Table 1: Excel spreadsheet page showing bioreactor geometrical ratio and the calculated vessel dimensions

Bioreactor geometrical ratio		
Ratio	Definition	Value
HT/DT	Height to diameter of the tank ratio: Aspect ratio	2
h/HT	Height of the liquid to height of the tank ratio.	0.8
d/DT	Diameter of the impeller to diameter of tank ratio	0.33
B/DT	Baffles width to diameter of the tank ratio.	0.1
Y/d	Clearance between the impellers to the diameter ratio	1.25
L/d	Height of the impeller blade to the diameter ratio.	0.2
W/d	Width of the impeller blade to the diameter ratio.	0.25
S/d	Clearance between the middle of impeller blade to the diameter ratio.	1
c/DT	Clearance between the baffles and the tank wall to the diameter of the tank ratio.	0.02
M/L	Distance between the middle of impeller to the height of the impeller ratio. Source: Doran (1995) each dimension is related to the vessel diameter, DT, as shown below	1
Dimension	Definition	x DT
DT	Diameter of the vessel	1
HT	Height of the vessel	2
h	Height of the liquid	1.6
d	Diameter of the impeller	0.33
B	Width of the baffle	0.1
y	Clearance between the impeller blades	0.413
L	Height of impeller blade	0.066
W	Width of impeller blade	0.083
S	clearance between the impeller and the bottom of he vessel	0.33
A	Height of the baffle	1.94
M	Distance between the middle of impeller blade	0.066
c	clearance between the baffle and the tank wall	0.198
The volume of the vessel, $V_T = \pi D_T^2 H_T / 4$		
Dimension	Definition	Value (m)
DT	Diameter of the vessel	0.15
HT	Height of the vessel	0.3
h	Height of the liquid	0.24
d	Diameter of the impeller	0.0495
B	Width of the baffle	0.015
y	Clearance between the impeller blades	0.06195
L	Height of impeller blade	0.0099
W	Width of impeller blade	0.01245
S	clearance between the impeller and the bottom of he vessel	0.0495
A	Height of the baffle	0.291
M	Distance between the middle of impeller blade	0.0099
c	clearance between the baffle and the tank wall	0.0297

Baffles are vertical strips of metal mounted against the wall of the tank to reduce vortexing or swirling of liquid. The optimum baffle width depends on the impeller design and fluid viscosity and often of the order $\frac{1}{10} - \frac{1}{12}$ of the tank diameter (Doran, 2005).

The agitation system dimensions were calculated with spreadsheet by linking with the cells of the vessel dimensions (Table 2).

2.4 Determination of the power required for agitation

Usually electrical power is used to drive impellers in stirred vessels. For a given stirrer speed, the power required depends on the resistance offered by the fluid to rotation of the impeller (Doran, 1995). Power required to operate a stirred tank is mostly the mechanical power required to rotate the stirrer. When estimating the stirrer power requirements for non-newtonian liquids, correlation of the power number versus the Reynolds number for Newtonian liquids are very useful (Katoh and Yoshida, 2009). Such

correlations are available in the literature (Aerstin and Street, 1978; McCabe et al., 1993; Doran, 2005; Katoh and Yoshida, 2009).

Table 2: Excel spreadsheet page for the agitation system dimensions

Definition	Ratio	value
Diameter of the impeller disc (Cd) to the diameter of the impeller	Cd/d	0.6
		Value (m)
Diameter of the impeller disc (Cd)		0.03
Blade width (W)		0.012
Blade Height (L)		0.01
Length of the shaft (Ls) should be grater than HT - S		> 0.25
The number of impellers (N)		3
Height of the baffle (A)		0.291

For ungassed system (McCabe et al., 1993; Doran, 2005; Katoh and Yoshida, 2009) the power required is given as:

$$P_w = \rho N_i^3 d^5 N_p \quad (2)$$

For the laminar regime,

$$Re_{ci} = \rho N_i d^2 / \mu_{fb} = 107.05 \quad (3)$$

where $N_i = 200 \text{ rpm} = 3.33 \text{ s}^{-1}$ (as used in the batch fermentation studies), $\rho = 9$ (maximum concentration of the fermentation broth from the experiment), μ_{fb} (viscosity of the fermentation broth from the literature $= 0.7 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$).

From the correlation of the power number, using the value of Re_{ci} calculated, the power number, $N_p = 5$ (Aerstin and Street, 1978), therefore, $P_w = 5.2 \times 10^{-4} \text{ W}$. The sheets containing the formula for the power number and dimensionless Reynolds number were linked with the vessel dimensions to calculate the value of the power factor (Table 3).

Table 3: Excel spreadsheet page for the power required for agitation

Reynolds number ($Nre = \rho N_i d^2 / \mu_{fb}$)	broth density = highest cell density $\text{kg/m}^3, \rho$	9
	impeller speed = 200 rpm N_i	3.33
	impeller diameter (m) d	0.05
	broth viscosity, μ_{fb} ($\text{kg m}^{-1} \text{ s}^{-1}$)	0.0007
		P_w (W)
Power required for agitation = shaft work (P_w)	Power factor (N_p)	5
$P_w = \rho N_i^3 d^5 N_p$		0.00052

2.5 Material and Energy Balance

In the design and operation of various bioreactors, a practical knowledge of physical transfer process, including both mass and heat transfer, is important (Katoh and Yoshida, 2009). Material and energy balance is very important in the mass and heat transfer analysis in the bioreactor design calculation. The material and energy balance was prepared as stated in the sections below using the spreadsheet. The kinetic data (Table 4) that are available from the previous experiments and other information (including chemical or physical properties obtained from the literatures) were linked with the cell from the stoichiometric spreadsheet. This cell was also linked to various relationships as stated in the sections below. Thence, the feed flowrate and the flowrate of ethanol in the product stream were calculated with the assumption that ethanol

concentration in the feed stream was zero. Likewise, with the assumptions that the system is at steady state, homogeneous system, isothermal condition and no heat loss due to evaporation, the energy balance spreadsheet was prepared using the stated information in the sections below. The cells of this spreadsheet were later linked with the cells of other spreadsheets for the calculations of the heat transfer equipment parameter as shown in Table 5.

2.5.1 Material balance

The stoichiometry of the fermentation reaction is given as:

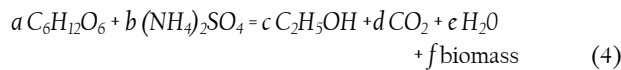


Table 4: The batch kinetic parameters from previous experiments

Medium	P_t	Y_{ps}	Y_{xs}	μ	Y_{etoh}	S_i	Q_p	Q_s	Y_{px}	Q
B1	48.16	0.47	0.07	0.11	92	1.45	1.98	4.38	5.22	0.6
B2	47.13	0.47	0.12	0.12	92	19.80	2.04	3.20	6.82	0.8
B3	55.28	0.49	0.05	0.11	96	10.52	1.94	4.98	9.00	1.0

Key:

B1 = Bioreactor runs using yeast extract as nitrogen source and 100 g/L CSH.

B2 = Bioreactor runs using ammonium sulphate as nitrogen source and 100 g/L CSH.

B3 = Bioreactor runs using 120 g/L CSH and ammonium sulphate as nitrogen source

The material balance on the batch fermentation system was carried out using the following information: 93 % fractional conversion of dextrose based on the concentration of glucose-rich hydrolysate fed (100 kg/m³), 100 % fractional conversion of (NH₄)₂SO₄, biomass yield from substrate (Y_{x/s}) of 0.08, ethanol yield from the substrate (Y_{p/s}) of 0.46, yield of CO₂ (Y_{co₂}) = 90 % (assumed).

2.5.2 Determination of the Flowrates of the Feed and Ethanol in the Product Stream

The volumetric flow rate of the feed

$$F_w = V_T (\% \text{ working volume, } V_{wr}) \times D_{opt} \quad (5)$$

$$D_{opt} = \mu_{max} \left[1 - \sqrt{\frac{K_s}{K_s + S_i}} \right] \quad (6)$$

μ_{max} is obtained from the Monod model:

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad (7)$$

The instantaneous value of μ is given by $\left(\frac{dx}{dt}\right) \frac{1}{x} = \mu$ and the values of K_s and μ_{max} were obtained from the plots of $1/\mu$ against $1/S$ for the data taken from the batch processes carried out in the bioreactor. The average value of D_{opt} of 0.082 was finally obtained. $V_{wr} = 0.8 \times 5 = 4 \text{ L}$

(using 80 percentage working volume). Therefore, $F_w = 3.28 \times 10^{-4} \text{ m}^3/\text{h}$. The mass flow rate of the feed, $M_w = F_w \times \rho_f$ (feed density) and for 100 g/L of the feed, $M_w = 3.28 \times 10^{-2} \text{ kg/h} = 9.11 \times 10^{-6} \text{ kg/s}$.

The ethanol flow rate in the product stream is given by:

$$E_w = \left(\frac{dp}{dt}\right) V_T (\% \text{ working volume, } V_{wr}) \quad (8)$$

$$\left(\frac{dp}{dt}\right) = 1.98 \text{ g/L/h (obtained from the experiment),}$$

Therefore, $E_w = 8 \times 10^{-3} \text{ kg/h} = 2.2 \times 10^{-6} \text{ kg/s}$.

2.5.3 Energy balance and Temperature control: determination of the length of cooling coil.

Normally, in the design of a fermenter there must be adequate provision for temperature control which will affect the design of the vessel body (Stanbury et al., 2000). Heat will be provided by mechanical agitation and microbial activity. On a laboratory scale, little heat is normally generated and temperature is controlled by internal heating coils or a heating jacket through which water is circulated. To make an accurate estimate of heating or cooling requirements for a specific process it is important to consider the contribution factors.

An overall energy balance for fermentation during normal operation (Stanbury et al., 2000) can be written as eqn 9:

$$Q_{exch} = Q_{met} + Q_{ag} + Q_{gas} - Q_{acc} - Q_{eva} - Q_{sen} \quad (9)$$

The cooling requirements (jacket and/ or pipes) to remove the excess heat from a fermenter may be determined by eqn 10:

$$Q_{exch} = U A \Delta T_m \quad (10)$$

The assumptions made are: steady state, homogeneous system, isothermal condition, no heat loss due to evaporation and no heat accumulation in the system. Since, heat generation rate due to aeration for an aerobic system is zero, defines the total rate of heat loss to the surrounding

$$Q_{exch} = P_w - \Delta H r_x n \text{ (due to metabolic activities)} \quad (11)$$

The heat transfer surface area of the vessel

$$(A) = U A \Delta T_m / Q_{exch}$$

The mean temperature difference is obtained from:

$$\Delta T_m = \frac{Trgt - (Tc_{nti} - Tc_{nto})}{2} \quad (12)$$

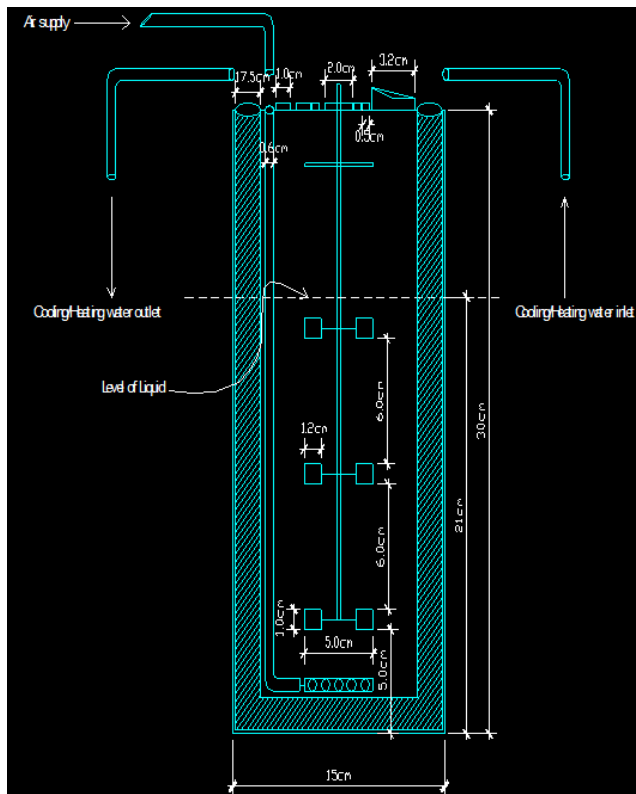
where $Trgt$ (Target temperature of the fermentation system) = 30 °C. The coolant is tap water available at 28 °C (Tc_{nti}).

abundance (cassava) to biofuel on a laboratory scale. The system could also be scaled-up for a pilot and industrial scale plant.

Table 6: Design summary and specification sheet

Project specification	Bioreactor/Fermentor Design	Raw materials	Quantity
Mode of Operation	Continuous/Semi-continuous	Microorganism (<i>S. cerevisiae</i>)	200 ml (inoculum)
Scale	Laboratory	CHS	400 g
Software application	Microsoft excel spreadsheet/HYSYS	(NH ₄) ₂ SO ₄	8 g
Material of construction	Stainless steel (with glass window)	feed flow rate	9.11EXP-6 kg/s
Shape	Cylindrical	EtOH flow rate	2.2EXP-6 kg/s
Total volume	5 Litres		
Working volume	4 Litres	Duty	
Head space volume	1 Litre	Heat exchanger	cooling coil
Aspect-ratio	2	Coolant	tap water
		fouling	1.7 KW/m ² /K
		Temperature	28 degree celcius
		Target temperature	30 degree celcius
		flow rate of the coolant	2EXP-6 m ³ /s
		overall coefficient of heat transfer	0.21 KW/m ² /K
		Heat transfer area	4.88 EXP-4 m ²
		Length of cooling coil	6.63 EXP-3 m
Agitation system			
Impeller type	Rushton turbine		
Num. of Impellers	3		
Num. of blade	6		
Num of baffles	4		
agitation speed	200 - 300 rpm		
power required for agitation	5.2 EXP-4		
agitator motor	AC/DC variable speed motor		
Probes			
pH	steam sterilizable gel-filled		
Temperature	steam sterilizable RTD		
Ancillary equipments			
pump	aquatic pump (1/2 H.P)		
water bath with pump			
mechaical seal/shaft			
air filter	hydrophobic teflon memebrane		

Fig. 2: AutoCAD drawing of the design bioreactor



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