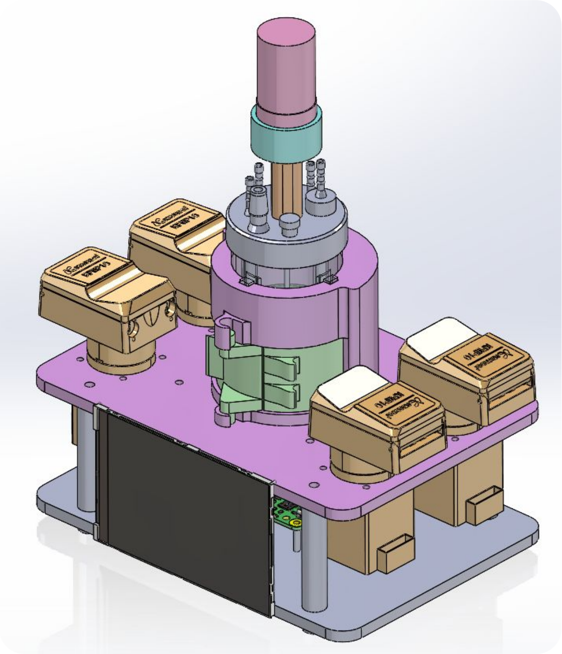
Non-Invasive Miniature Bioreactor for Laboratory Engineering (NIMBLE)



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What is a bioreactor?

- Vessel or tank that creates optimal environment for biological reactions
- Used for many applications
  - Cell culture
  - Fermentation
  - Biopharmaceutical production
- Come in many different sizes.

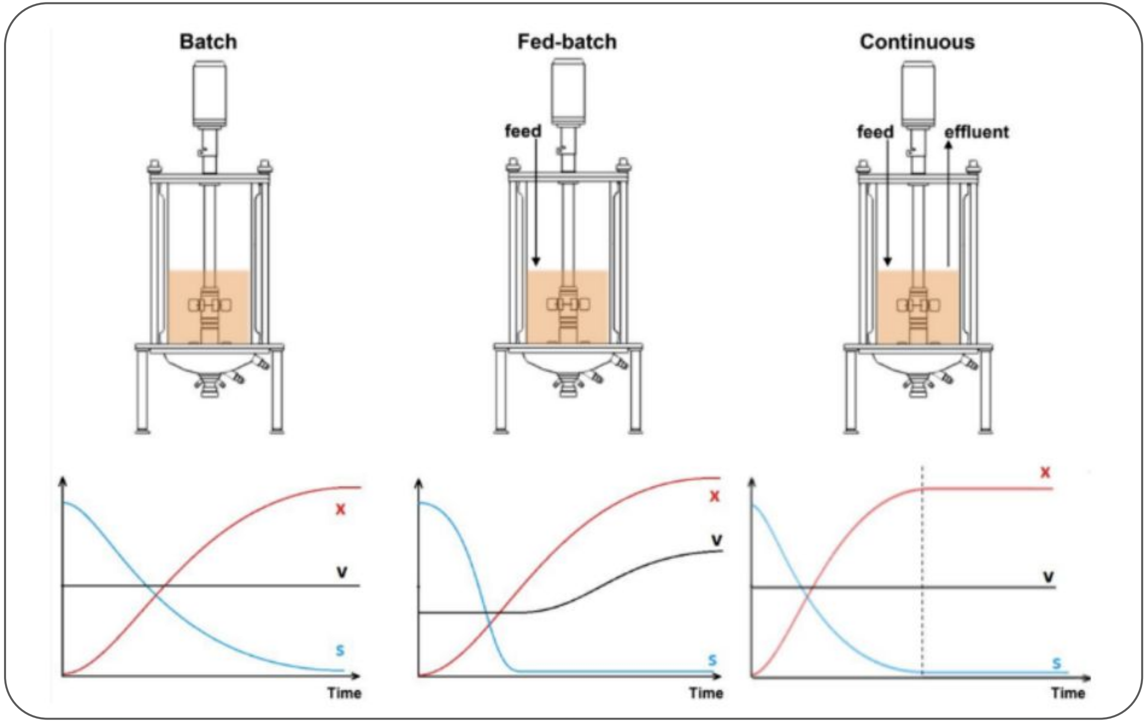
100mL-40L single-use bioreactors (Eppendorf)

250mL single-use bioreactor (Sartorius)

5L-30L reusable bioreactor (Sartorius)

Types of Bioreactors

- Batch
    - All nutrients added at the start of the process
- Fed-batch
    - Nutrients are added
    - No products are removed
- Continuous
    - Products are simultaneously removed
    - Higher population concentrations
    - Remove waste and product during reaction
    - Continuously add nutrients



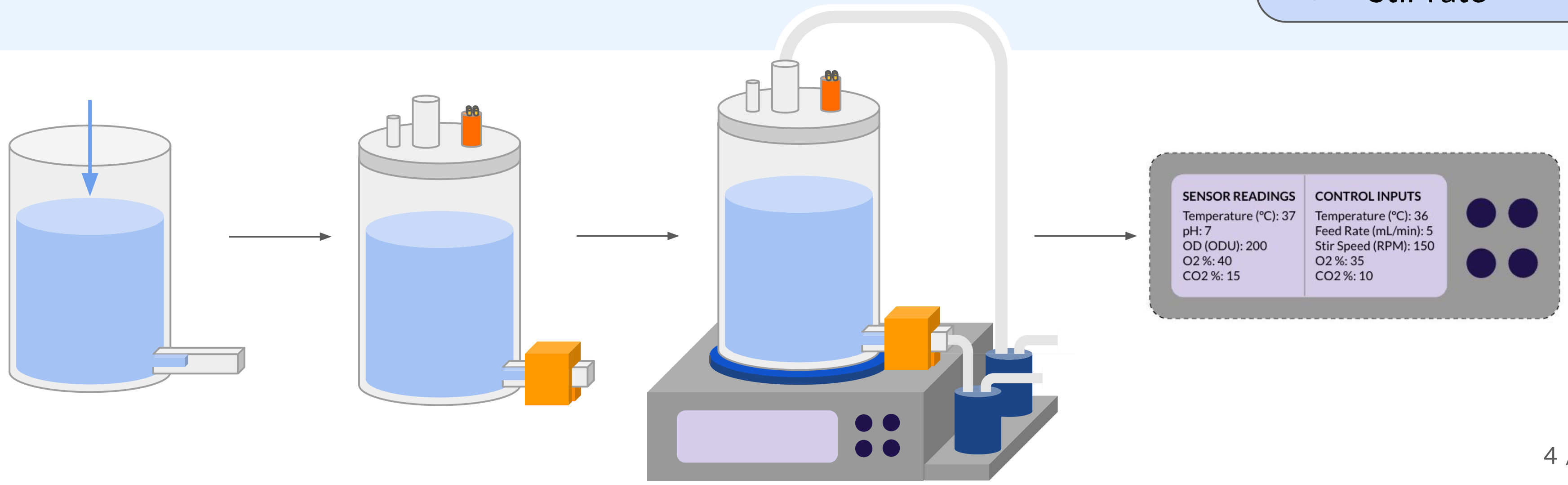
x = culture population v = culture volume s = substrate concentration

How NIMBLE works

Set Up

Add media to vessel → Assemble bioreactor → Calibrate sensors → Attach tubing → Attach feed, acid and base inputs → Input control parameters:

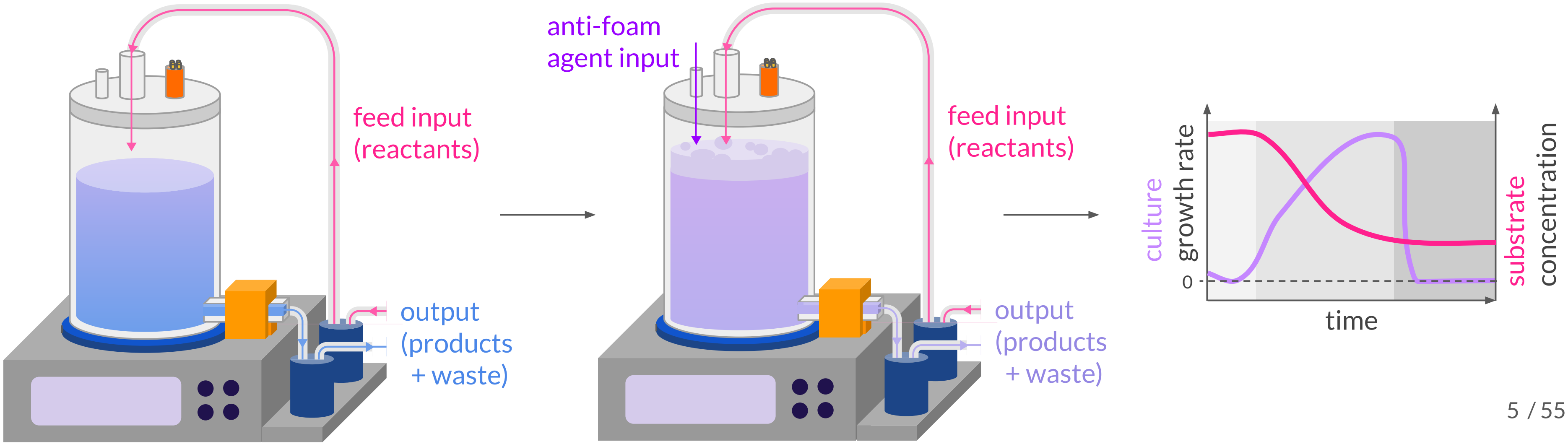
- Feed rates
- Target temp.
- Target pH
- Stir rate



How NIMBLE works

Run Reaction

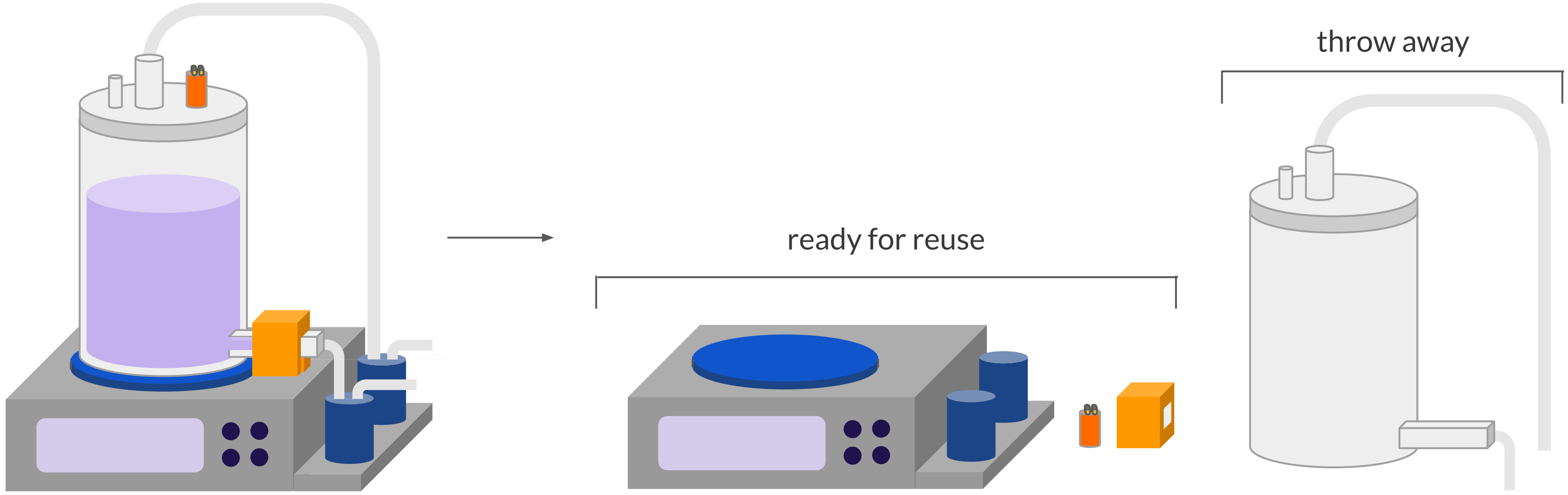
Start reaction → Read live sensor data on LCD screen → Inlet for anti-foaming agent → View logged data on SD card



How NIMBLE works

Clean Up

Stop reaction → Detach connections → Throw away disposable components → Bioreactor is now ready for next use!

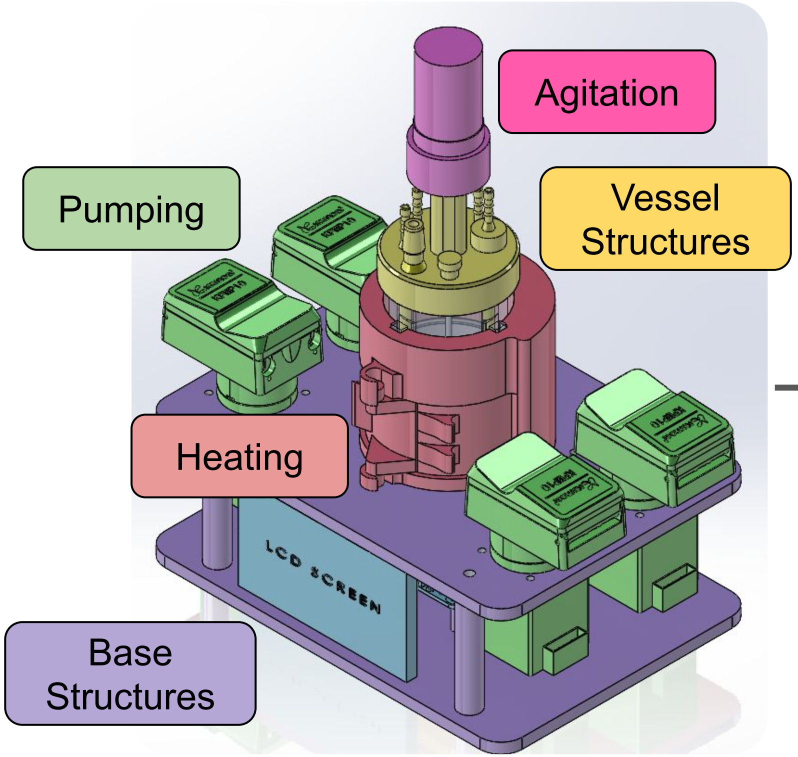


Our Niche

	Our Bioreactor	General Large-Scale	General Lab-Scale
Low-cost (reuse)	☑	☐	☐
Single Use	☑	☐	☐
Temp sensing and control	☑	☑	☑
pH sensing and control	☑	☑	☐
DO sensing and control	☑	☑	☐
OD sensing	☑	☑	☑
Perfusion Continuous Flow	☑	☐	☐
Chemostat Continuous Flow	☑	☐	☐
100% non-invasive sensing	☑	☐	☐
Lab-scale (~100mL)	☑	☐	☑
Data Logging	☑	☐	☐

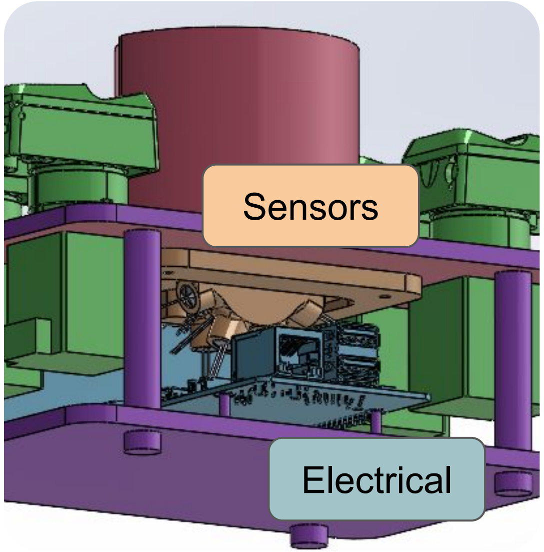
Have | Some have | Don't have

NIMBLE Assembly



Assembled Bioreactor

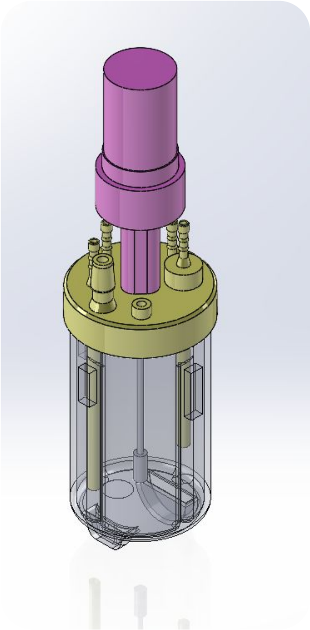
Reusable



Bioreactor Base

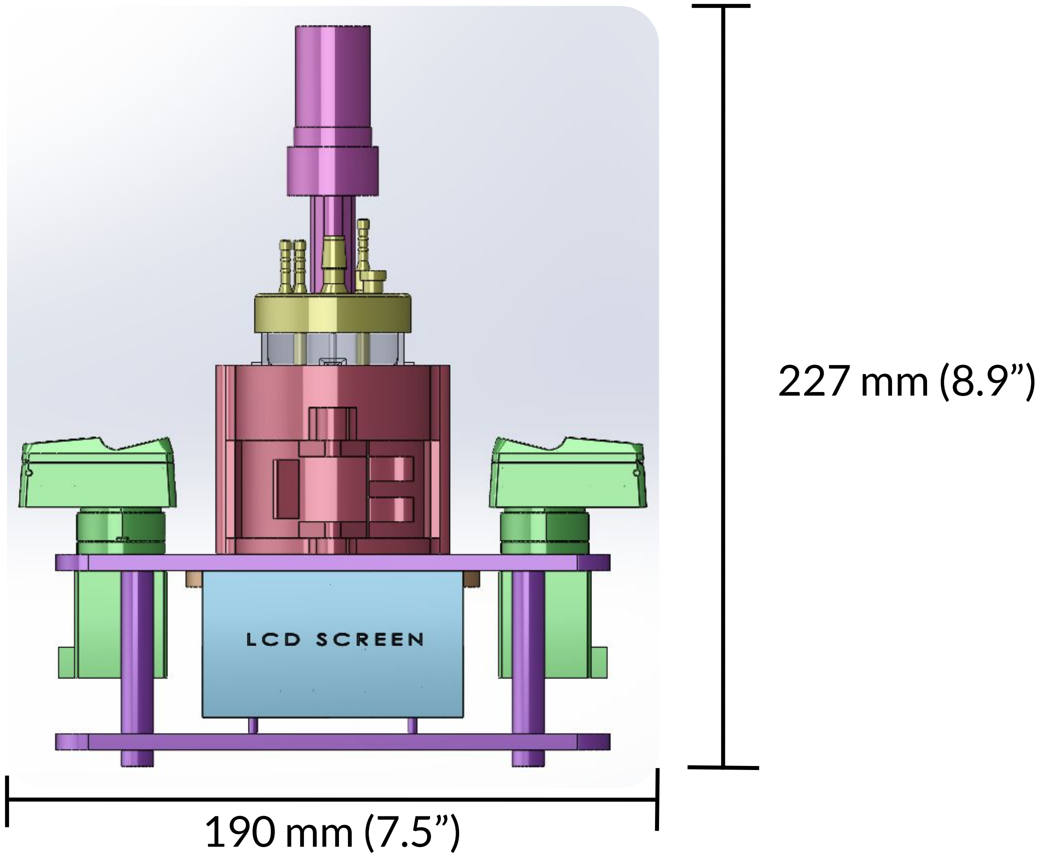
+

Disposable

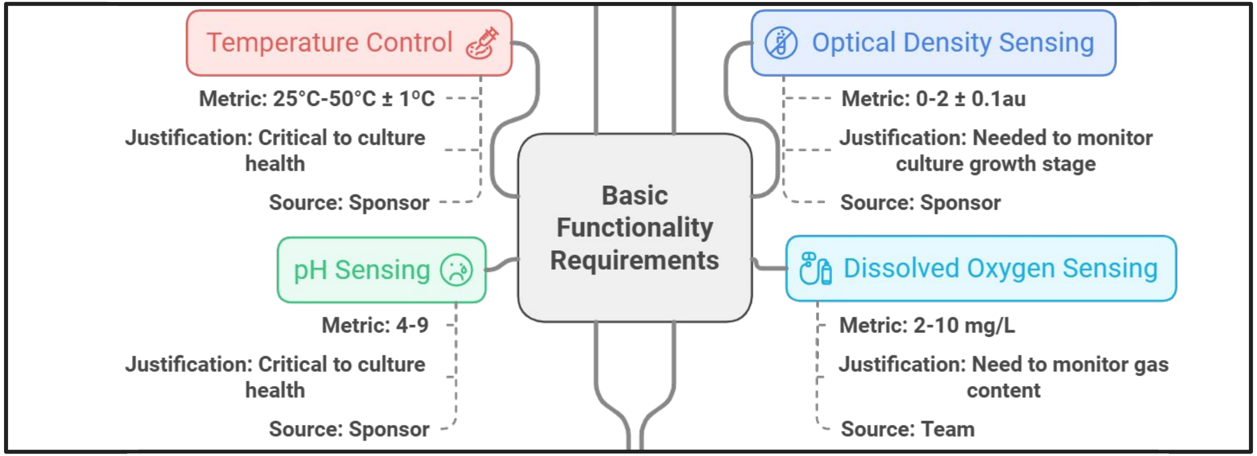


Reactor Vessel + Stirring Motor

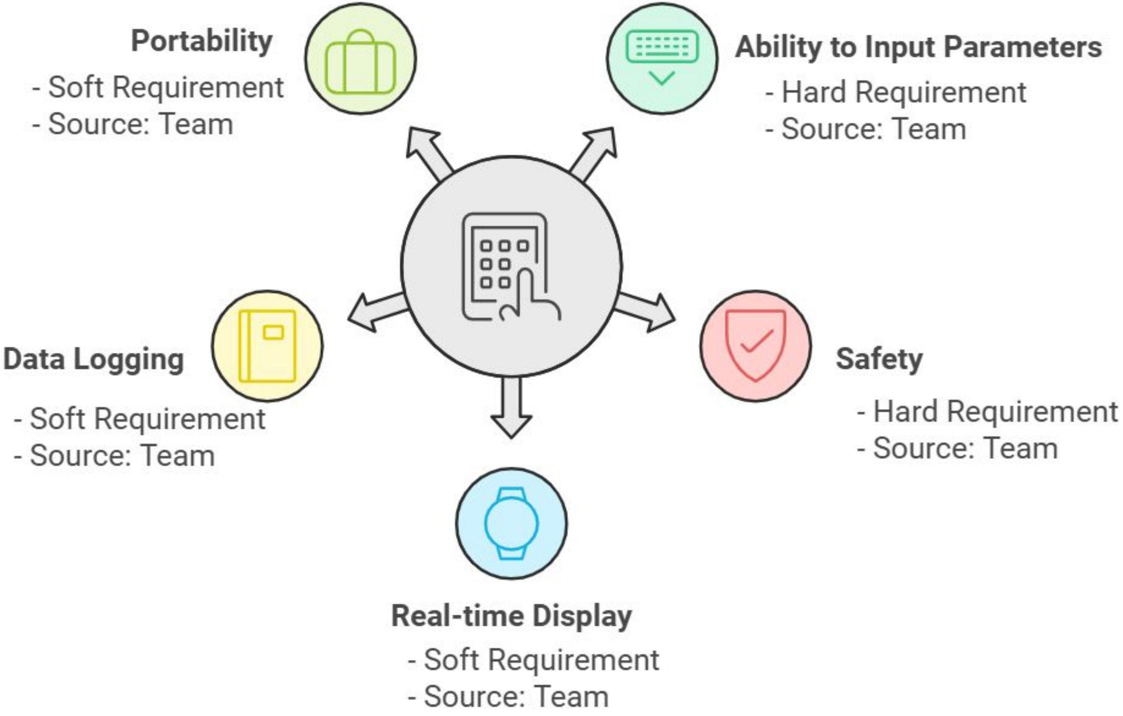
NIMBLE Assembly



8 Basic Design Requirements



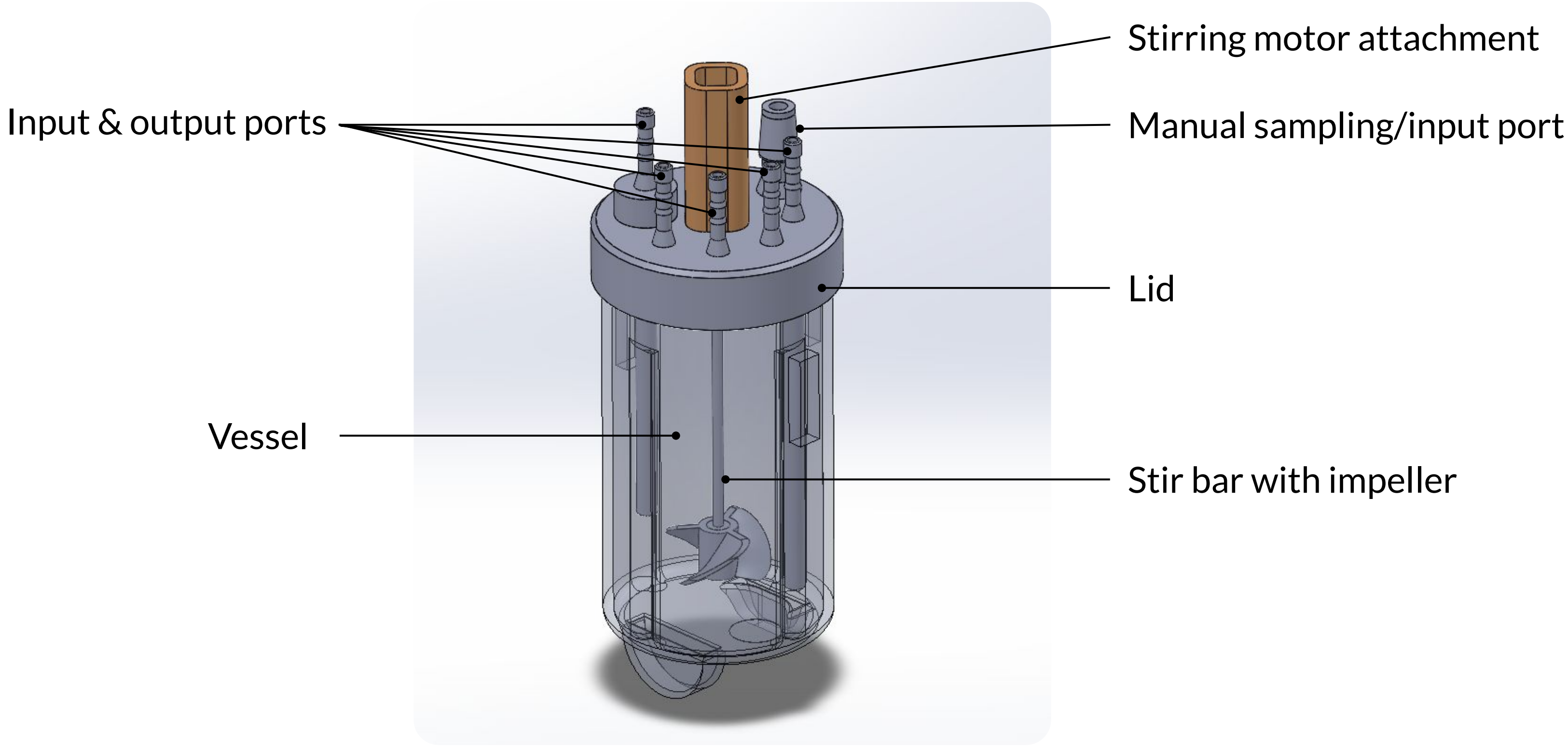
5 User Requirements



1 Cost Requirement

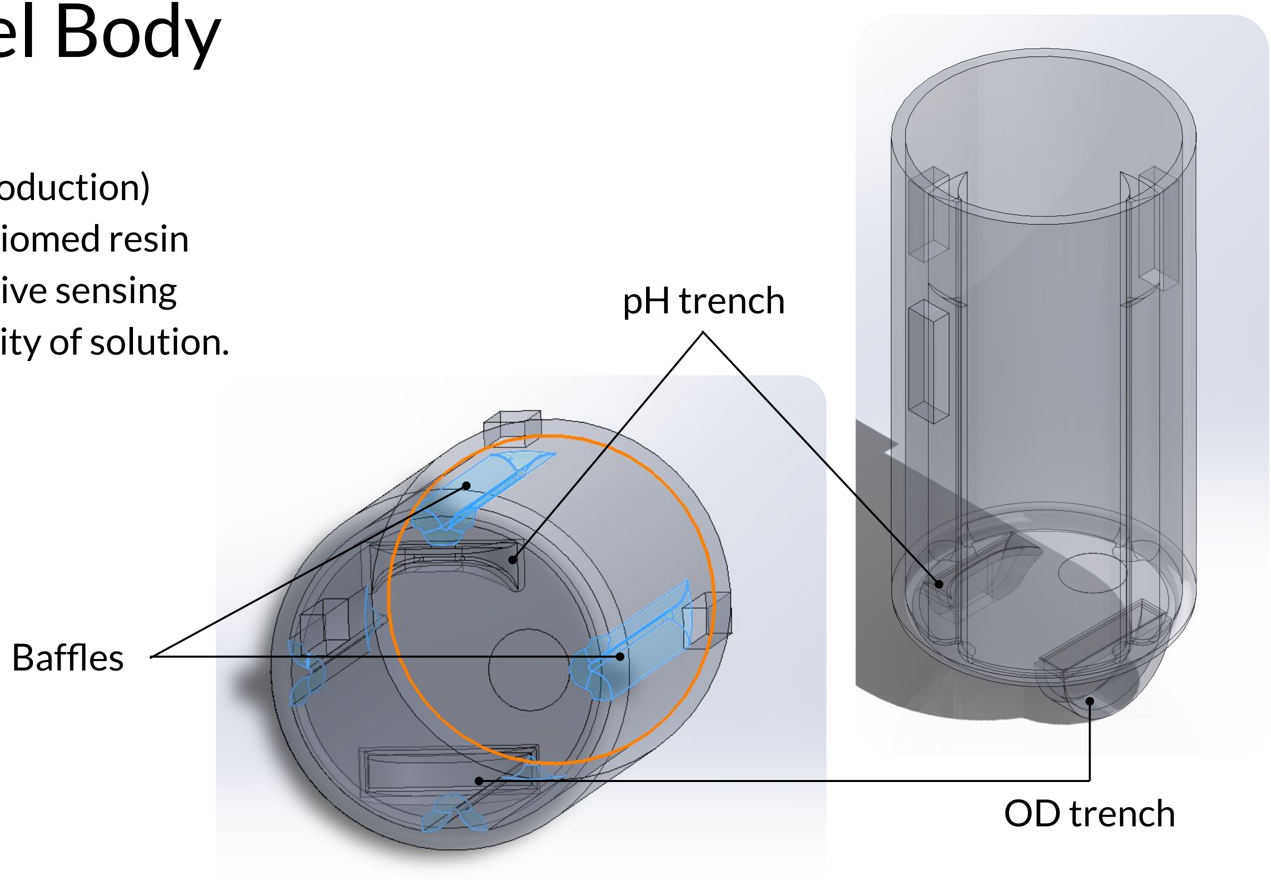
Cost (Hard Req.)		
Requirement	Metric	Source
Overall cost	<$1000	Team

Disposable Components



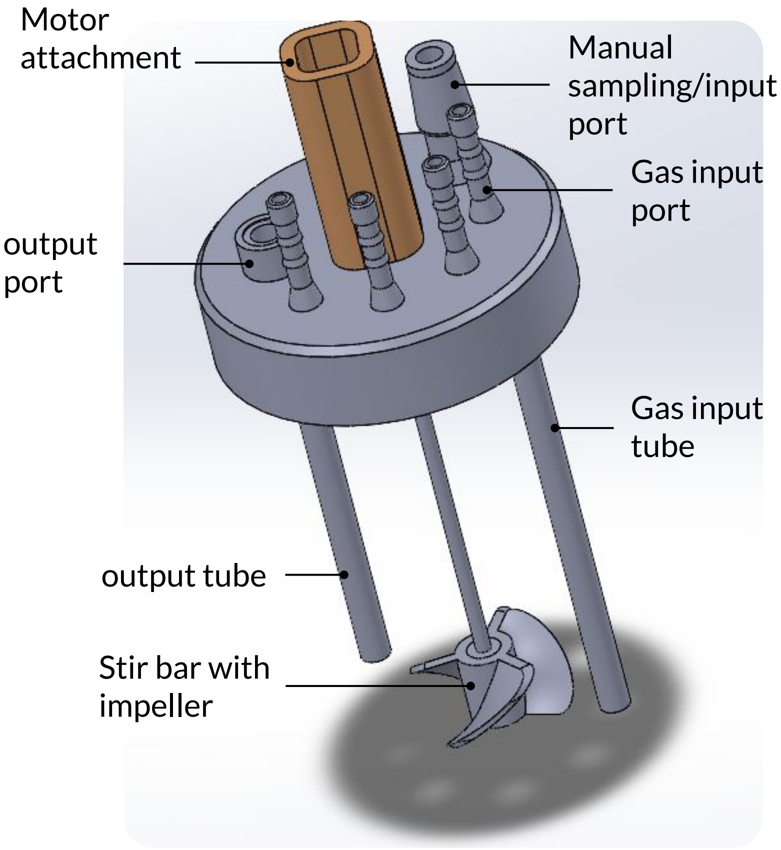
Innovative Vessel Body

- 100mL volume container
- Material: Nylon-12 (mass production)
    - Prototype: Formlabs biomed resin
- Trenches allow for non invasive sensing
- Baffled to ensure homogeneity of solution.

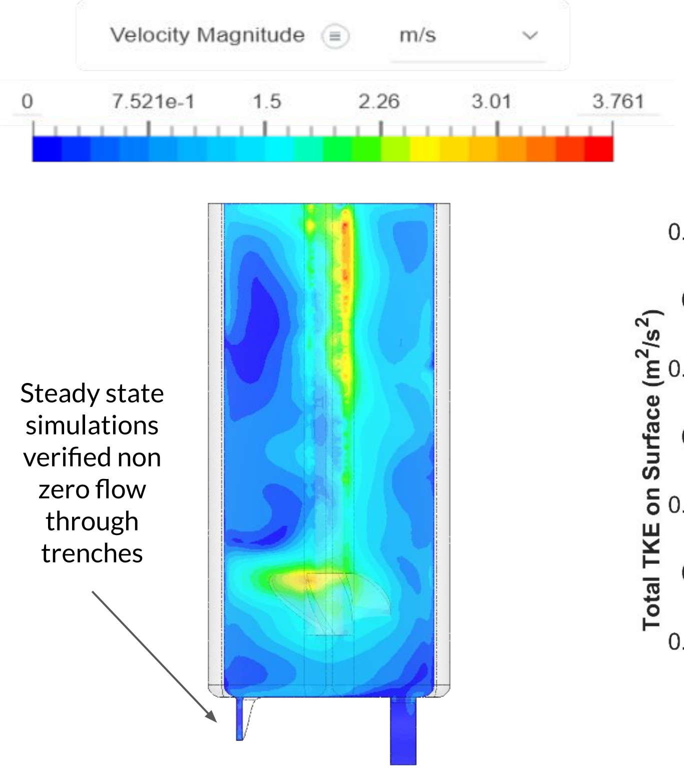


Vessel Lid

- Inputs and outputs for functional requirements
- Output draws from mid-vessel
- Internal mixer
  - Keyed interface for motor mounting
  - Motor coupled to stir bar through the lid
- 2 options (decided by user)
  - Option 1: PVDF membrane (filter) in the output
  - Option 2: no filter



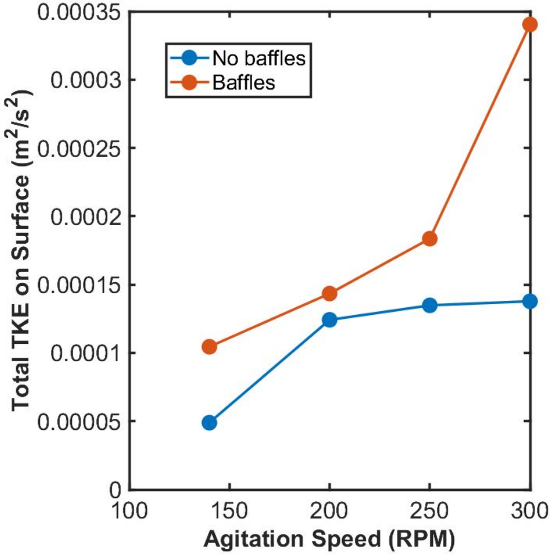
Innovative Agitation Design



Steady state simulations verified non zero flow through trenches

Example run at 300 RPM

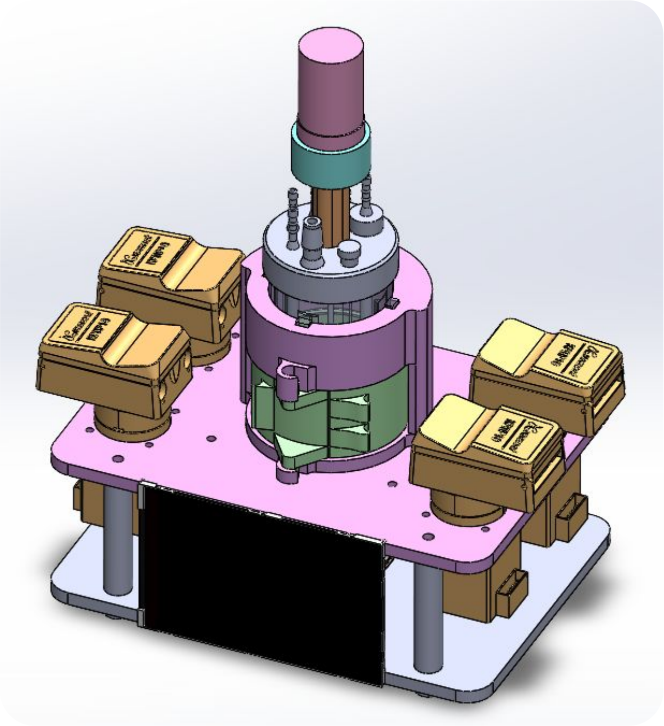
Total turbulent kinetic energy before vs after baffles included



Oxygen Transport (at 300 RPM)
without Gas Input

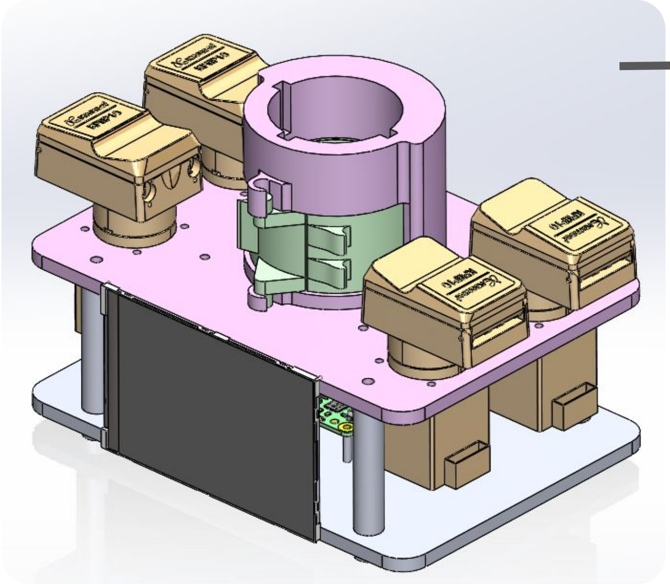
Significant gradient highlights need for gas input

Reusable Components

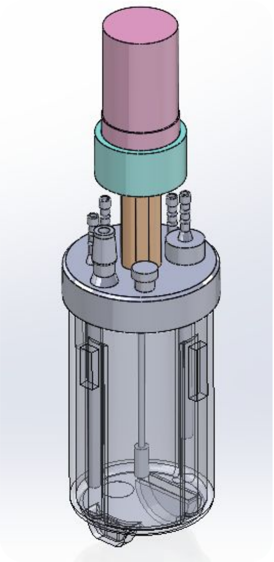


Assembled Bioreactor

Reusable Components



Bioreactor Base

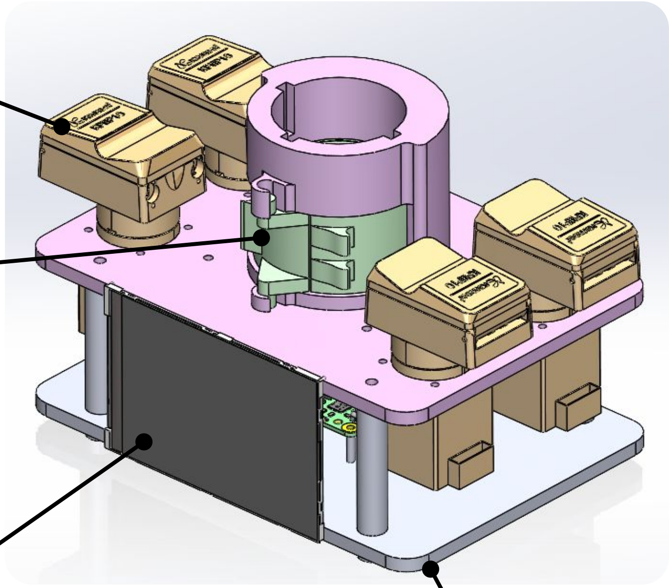


Reactor Vessel + Motor

Reusable Components

Pumps

Heating sleeve

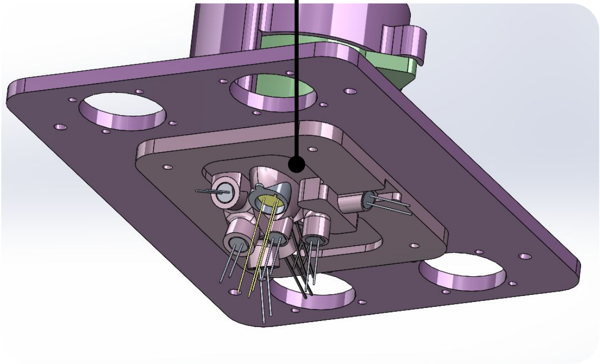


LCD Screen

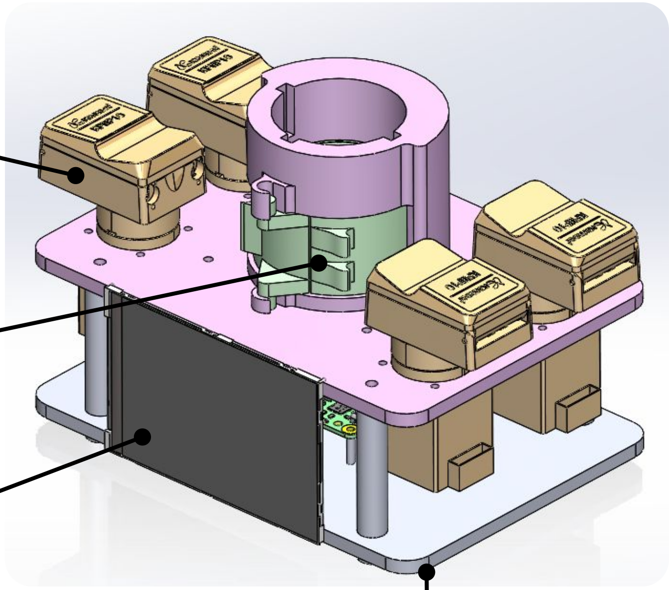
Primary structure

Stirring motor (not pictured)

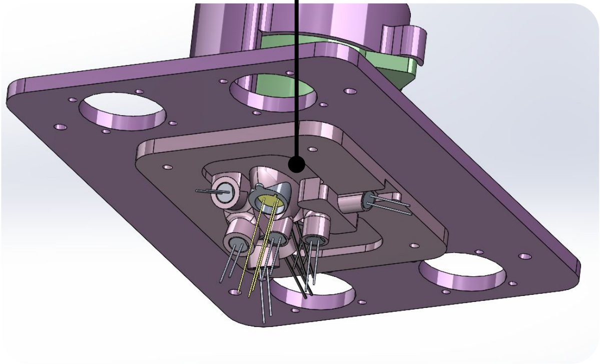
Sensor package



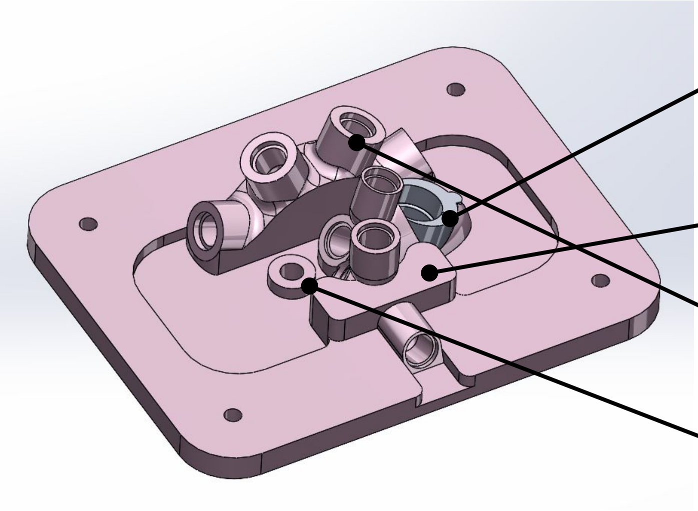
Reusable Components



Stirring motor (not pictured)



Innovative 4-Sensor Setup



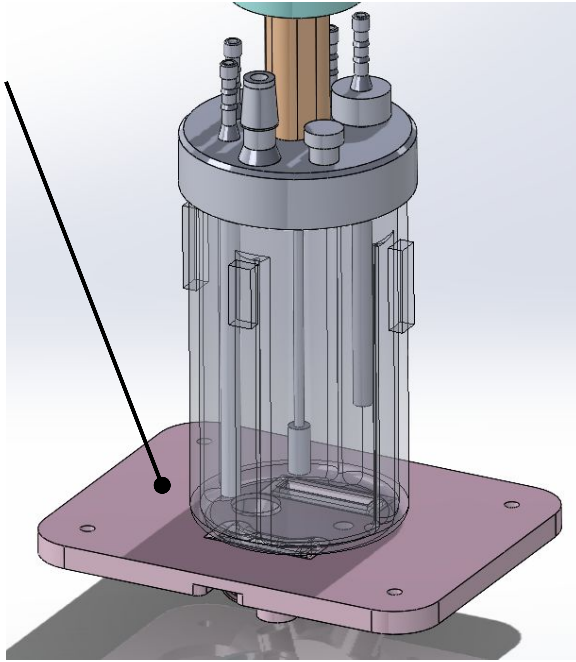
Sensing Platform Dimensions: 60x80mm

Dissolved Oxygen **(DO)** Sensor

pH Sensing Channel

Optical Density **(OD)** Sensor

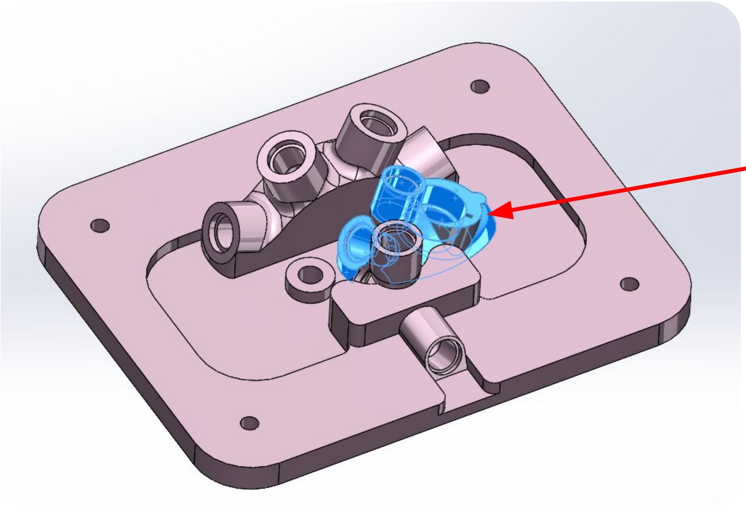
Thermistor location



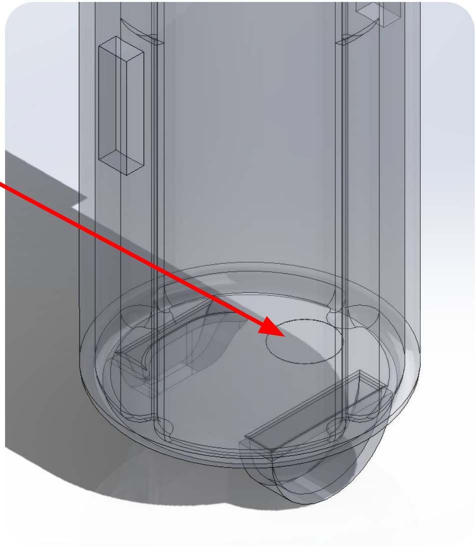
Sensor Package Exterior: Bottom View

Reaction Chamber: Side View

Dissolved Oxygen (DO) Sensor



Dissolved Oxygen (DO) Sensor



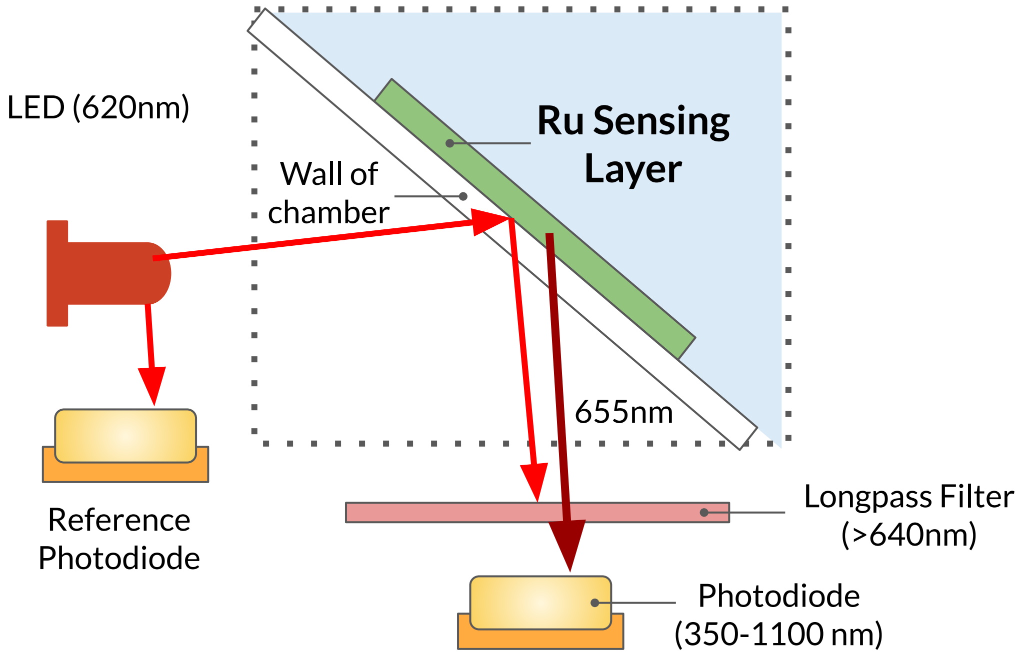
Sensor Package Exterior: Bottom View

Reaction Chamber: Side View

DO Sensor

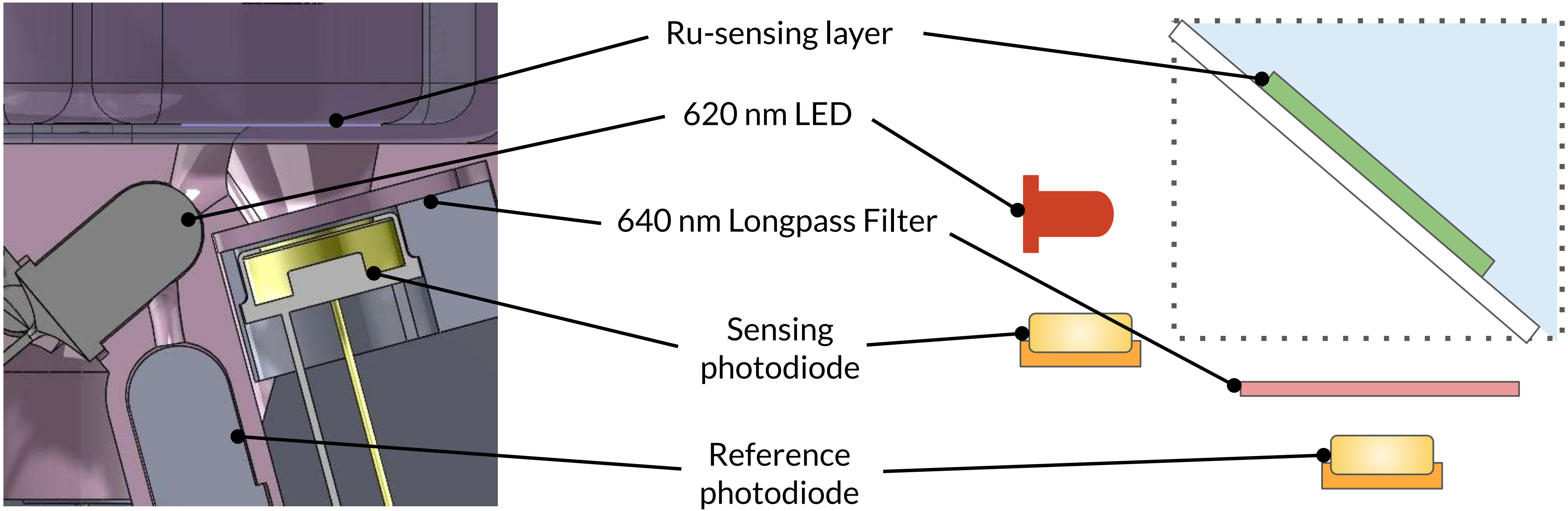
spec: 2-10 mg/L ± 0.2 mg/L

Dissolved Oxygen Sensing Schematic [2]



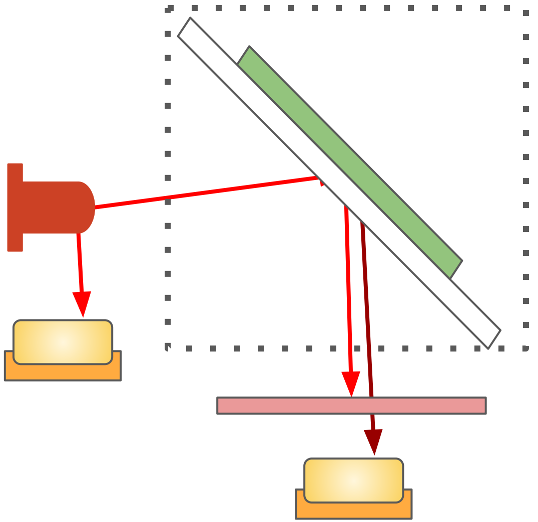
Similar to as demonstrated by [1]

DO Sensor Package

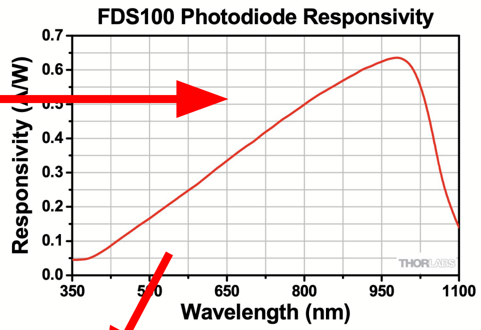


DO Sensor: Cross Section

DO Sensor Model



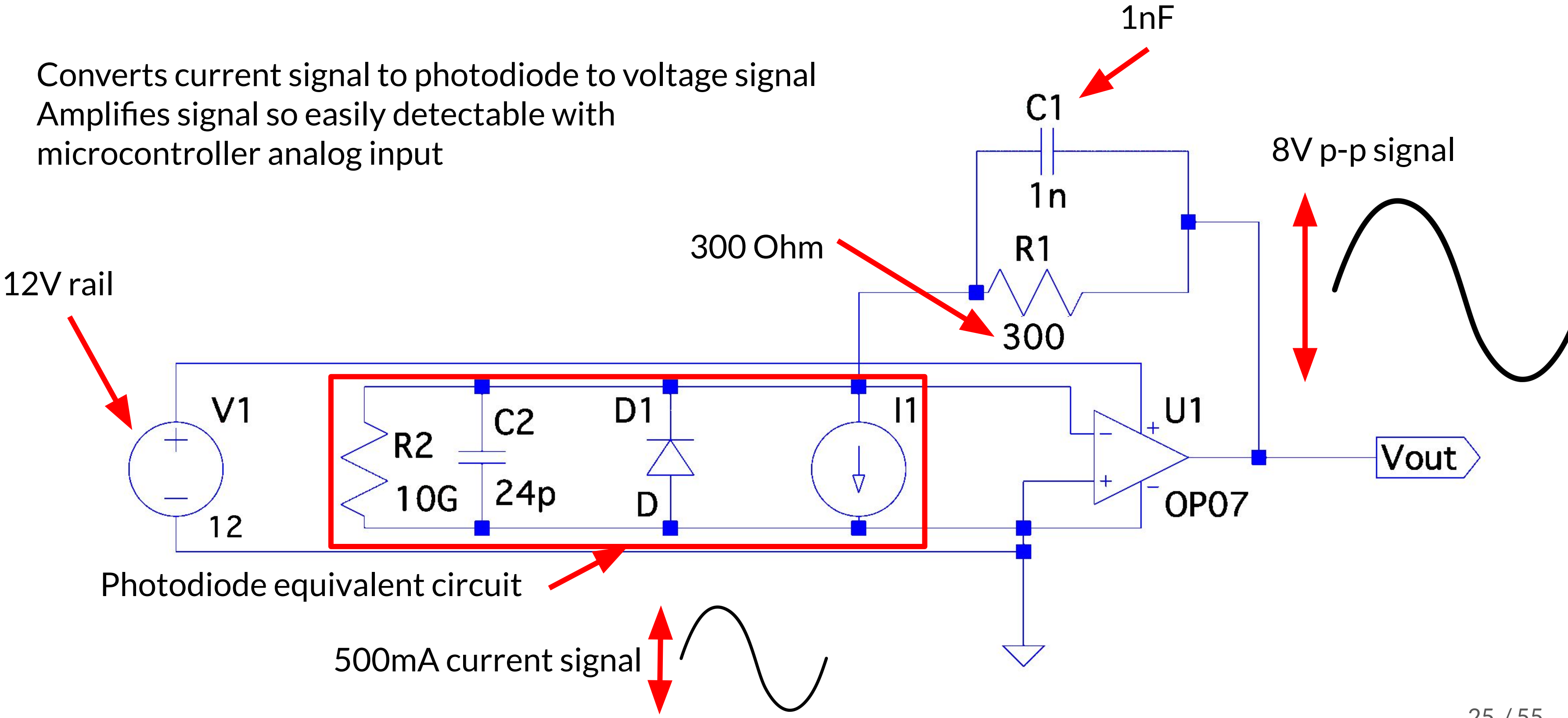
~1.44mW of light incident upon the photodiode



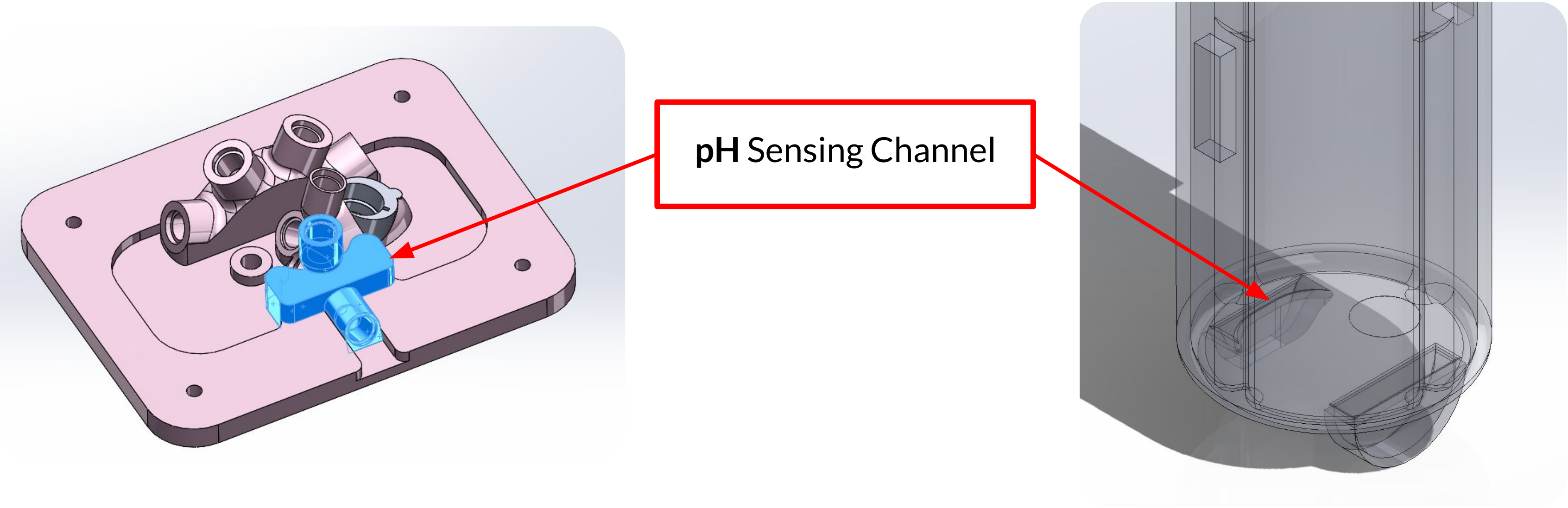
0.35*1.44 = 0.504mA of generated current in the photodiode

DO Amplifier Circuit

1. Converts current signal to photodiode to voltage signal
2. Amplifies signal so easily detectable with microcontroller analog input



pH Sensing Channel

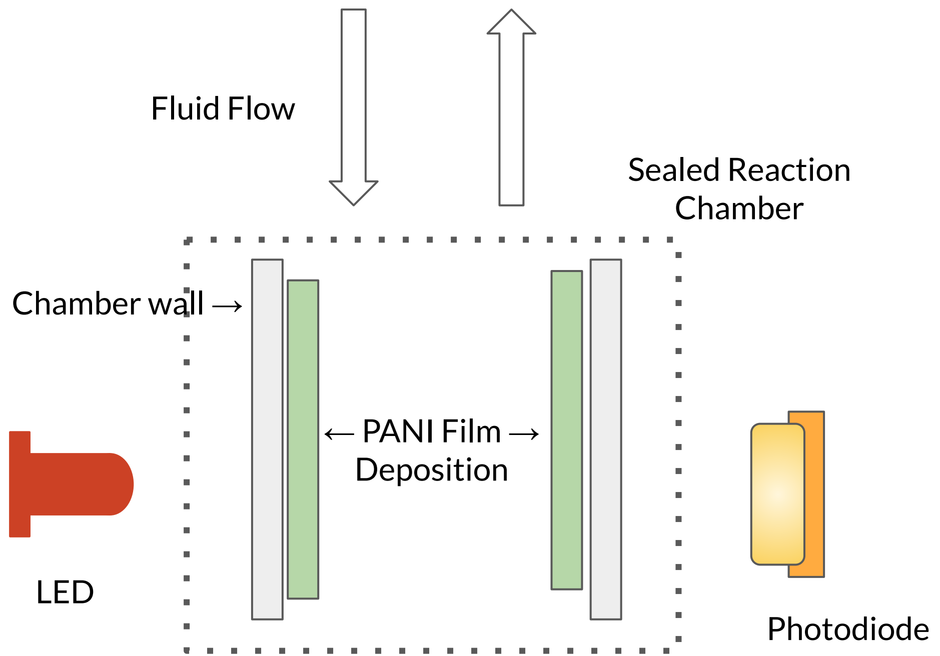


Reaction Chamber Exterior: Bottom View

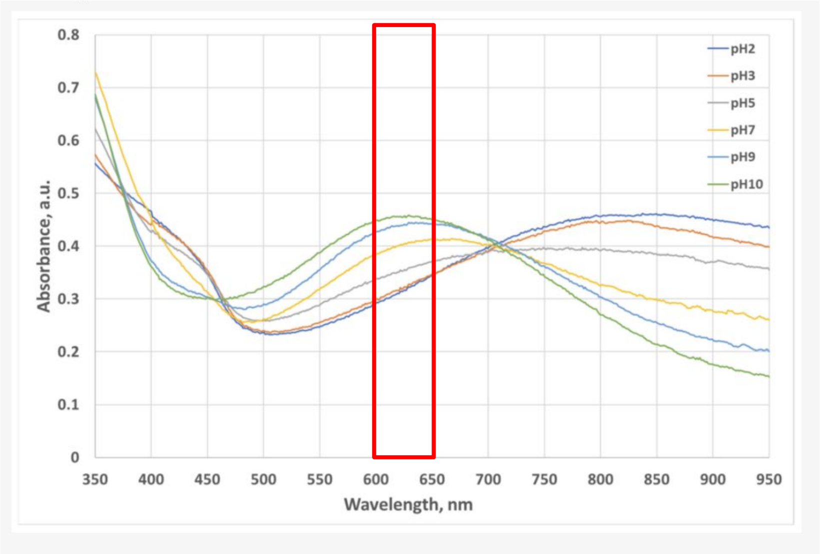
Reaction Chamber: Side View

pH Sensor

spec: 4-9 ± 0.1



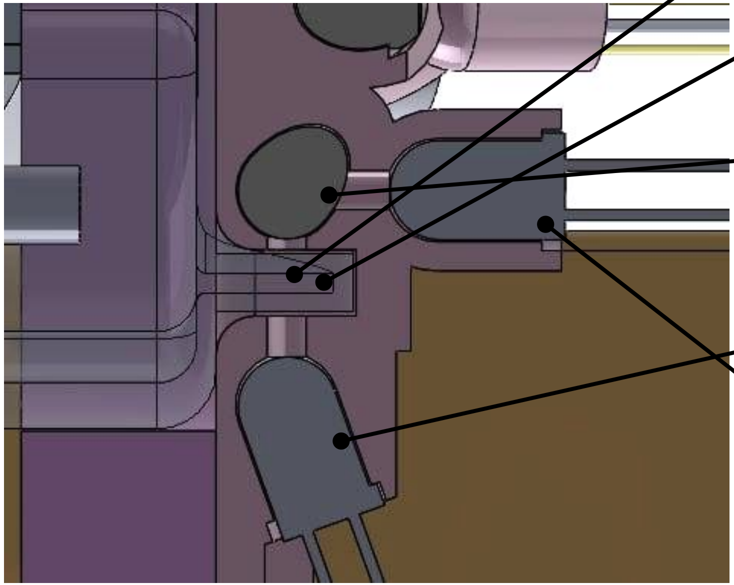
pH Sensing Diagram



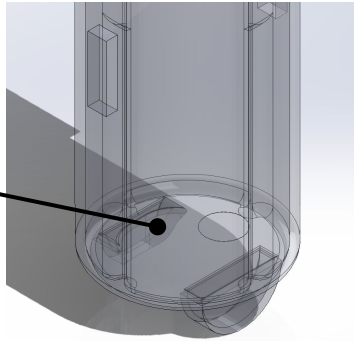
Absorption of an 8 um thick PANI film [3]

Fundamental Mechanism:
pH Dependent Absorbance of Polyaniline

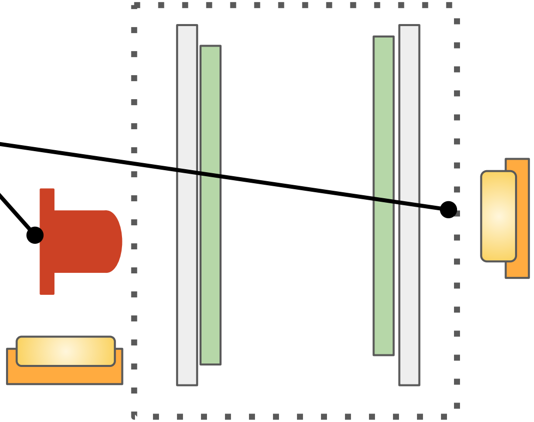
pH Sensor Design



Cross section



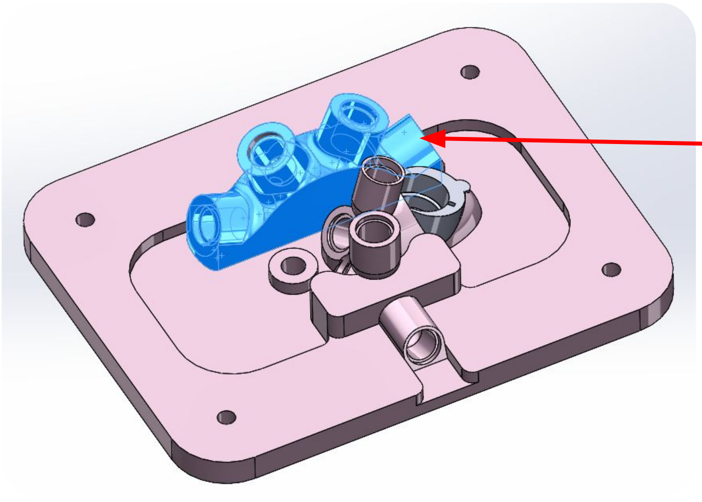
Vessel Side View



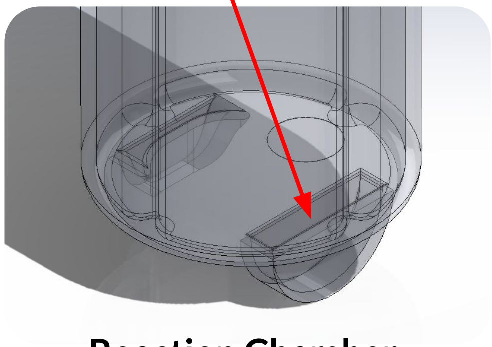
pH Sensing Diagram

Optical Density (OD) Sensor

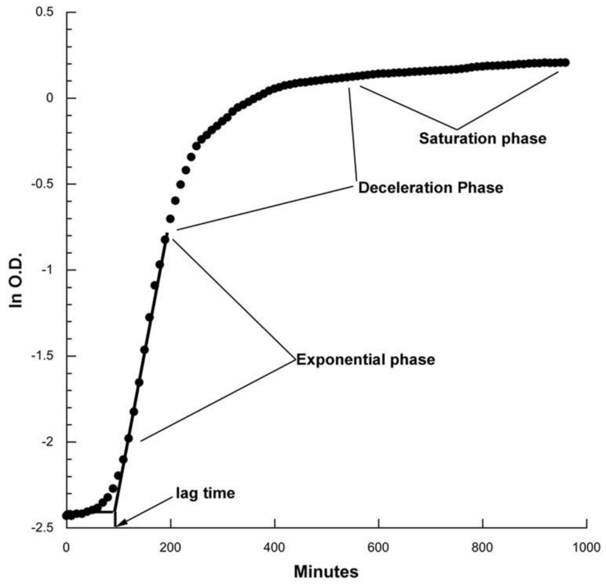
Optical Density **(OD)** Sensor



Reaction Chamber Exterior: Bottom View



Reaction Chamber: Side View

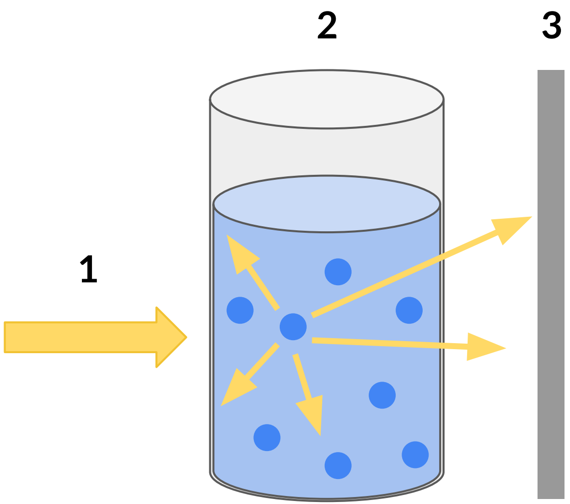


Typical Bacterial Growth Curve

OD Sensor

spec: 0-2 ± 0.1 au

1 Emit light (λ = 600nm) → **2** Light scatters → **3** Detector measures transmitted light → **4** Calculate OD: OD = $\log_{10}(I_0/I_t)$



Scatter Angle

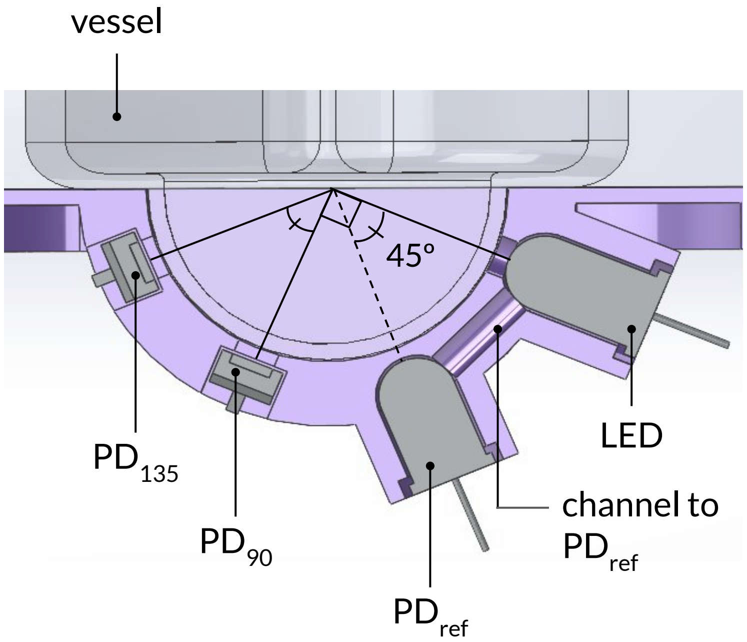
- Larger angle, low density
  - More sensitive
  - Saturates quicker
- Smaller angle, high density
  - Saturates quicker
- Optimal: 45°, 90°, 135°

Normalization

- LED intensity dims over time
- LED intensity is sensitive to surrounding temperature
- Measure initial LED intensity with reference photodiode (PD)

Similar to Pioreactor (competitor bioreactor)

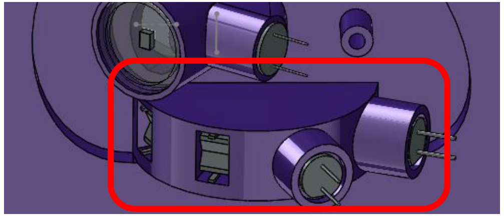
OD Sensor Design



OD Sensor: Cross Section

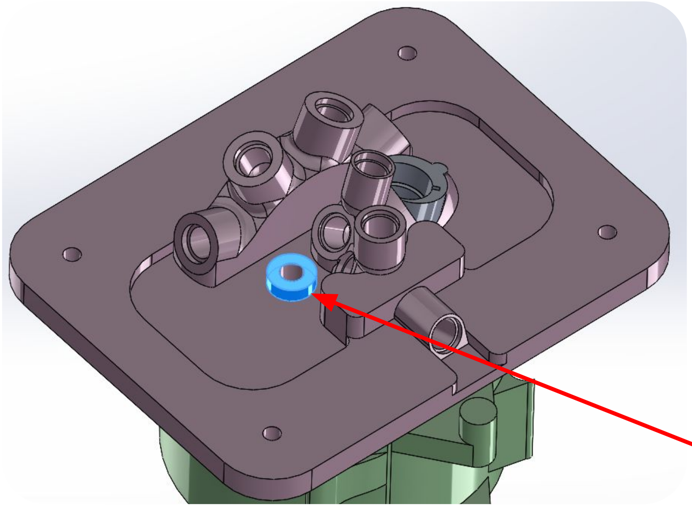
Cost breakdown + Component Selection

Part	P/N	Supplier	Cost
LED	521-9704F	Mouser	$0.67
Reference PD	SFH 213 FA	Mouser	$0.60
2x Measuring PD	1621132	Jameco	$0.95
2x amplifier circuits	Slide 19/20	Slide 19/20	Slide 19/20

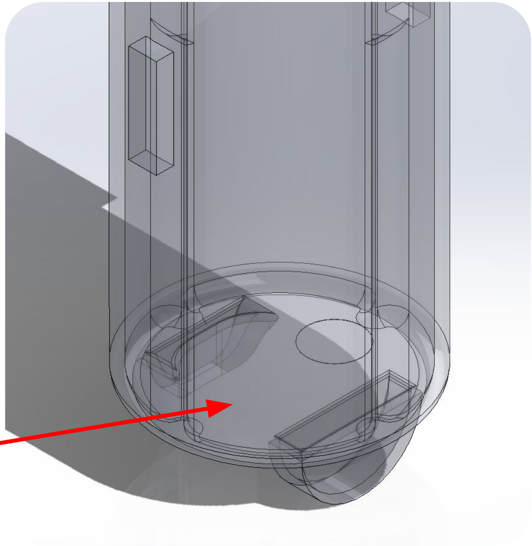


OD Placement: Bottom View

External Thermistor



Thermistor location

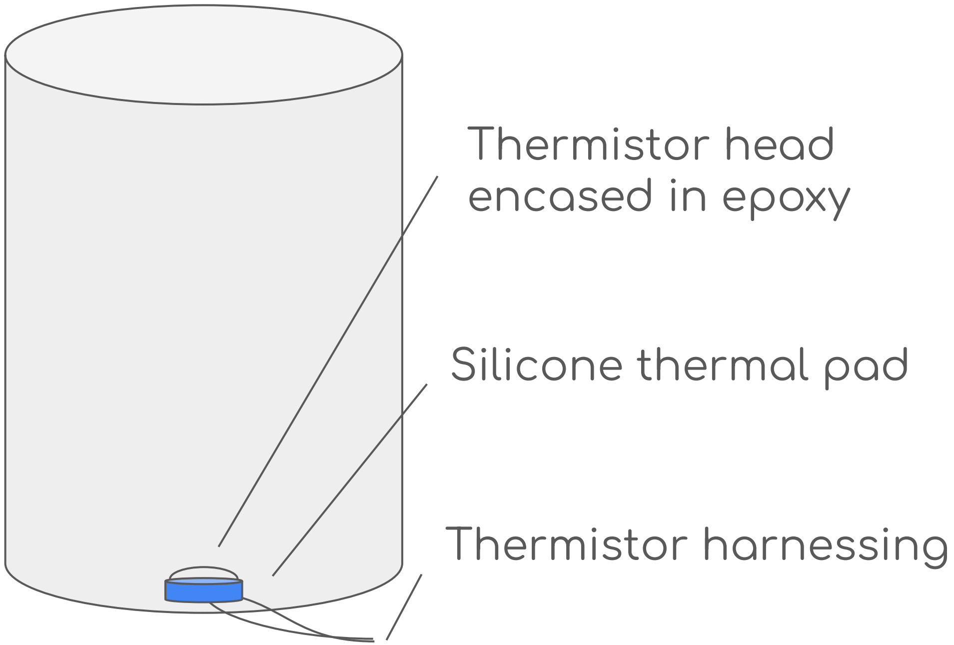


Reaction Chamber Exterior: Bottom View

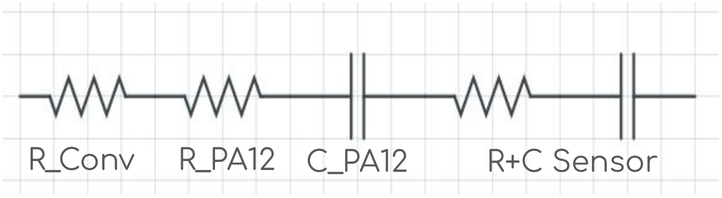
Reaction Chamber: Side View

Thermistor Temperature Validity

spec: 25-50°C ± 0.5°C



Thermal Network

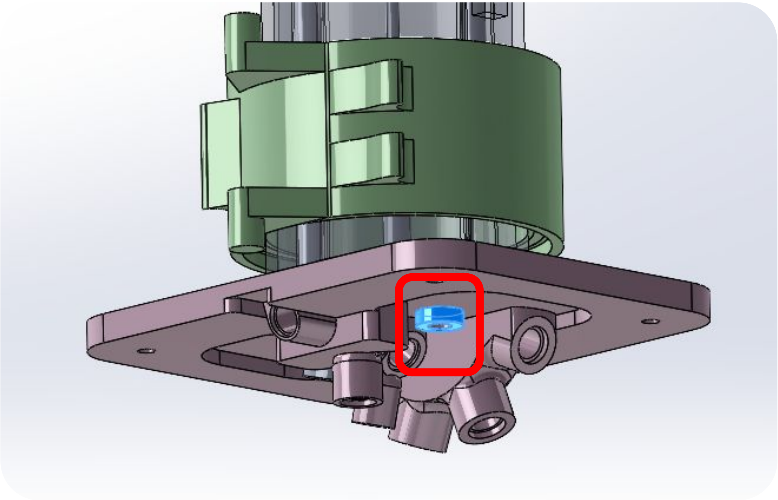


Estimated sensing time constant: ~30 seconds

Temperature Sensing Requirement: <30 seconds

Similar to Pioreactor (competitor bioreactor)

Temperature Sensor Design



Thermistor Placement: Bottom View

Cost breakdown + Component Selection

Part	P/N	Supplier	Cost
Thermistor	NRCE154K 4200B1KS	Mouser	0.17
Thermal pad	B0BD5PJ6 FL	Suspirit	0.02

Heater Selection

12.7 x 2.54 cm 0.78W/cm^2 Flexible Silicone Rubber heat pad

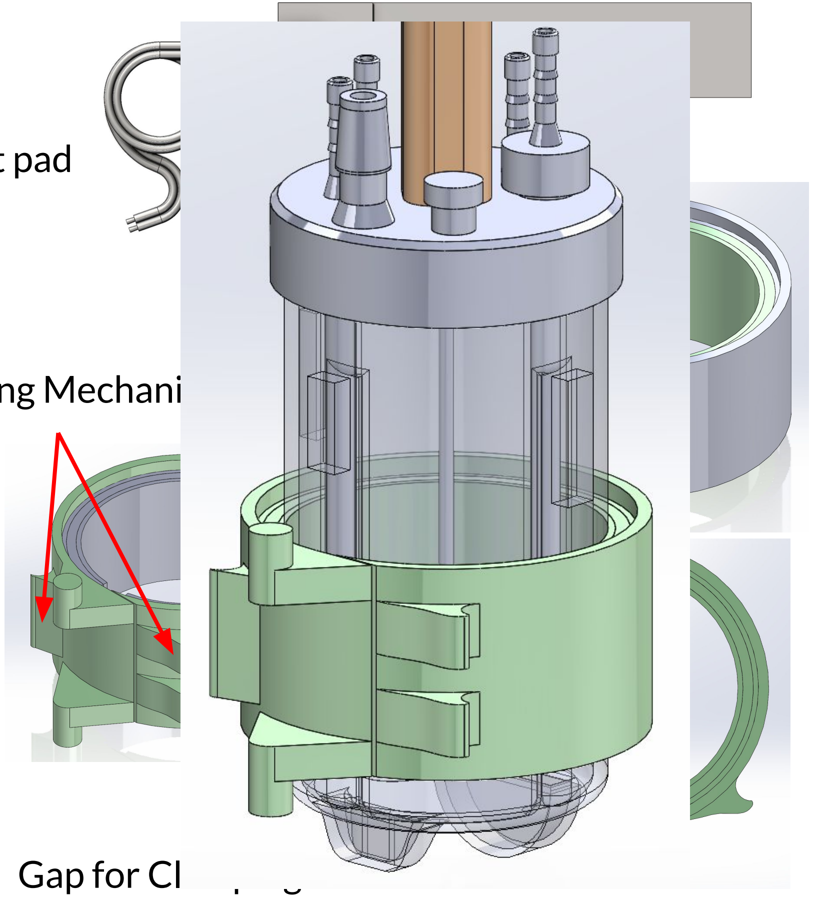
Requirement	Metric
Temperature control	25°C-50°C ± 1°C
Optical density sensing	0-2 ± 0.1au
pH sensing	4-9
Leak and ingress proof	IP68

$$q = mc_w \Delta T \rightarrow 12,552J \approx 12kJ$$

- To heat to max in 10 min need 20W
- Heat pad can supply 25W total
- Clamped near the bottom of the vessel

$$\frac{\text{Heated SA}}{\text{Volume}} \rightarrow \frac{32cm^2}{100cm^3} = 0.32cm^{-1}$$

Clamping Mechani



Gap for Cl

Heater Thermal Insulation

Flexible Teknofibra 2mm Adhesive Sheet

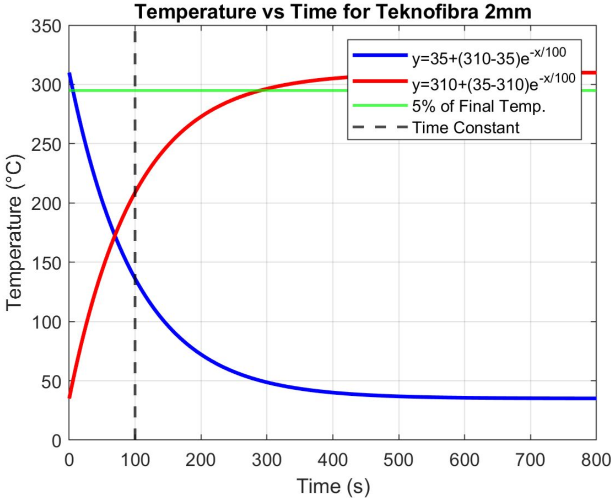
Hard/Soft	Requirement
Hard	Ability to input parameters
Hard	Safety
Soft	Real-time display of parameters
Soft	Data logging
Soft	Portable

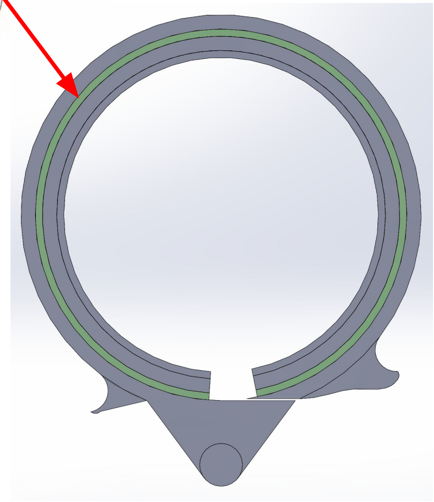
From Teknofibra Test Report:

- 300s Temp. Stabilization for ΔT= 275°C
- Assuming within 5% of final: τ=100s

$$T_{out} = T_{in} - \dot{Q}R_{wall}$$

Temperature @ External Wall: 38°C



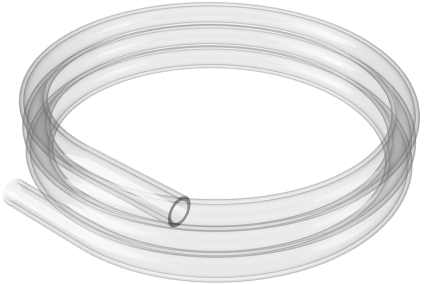


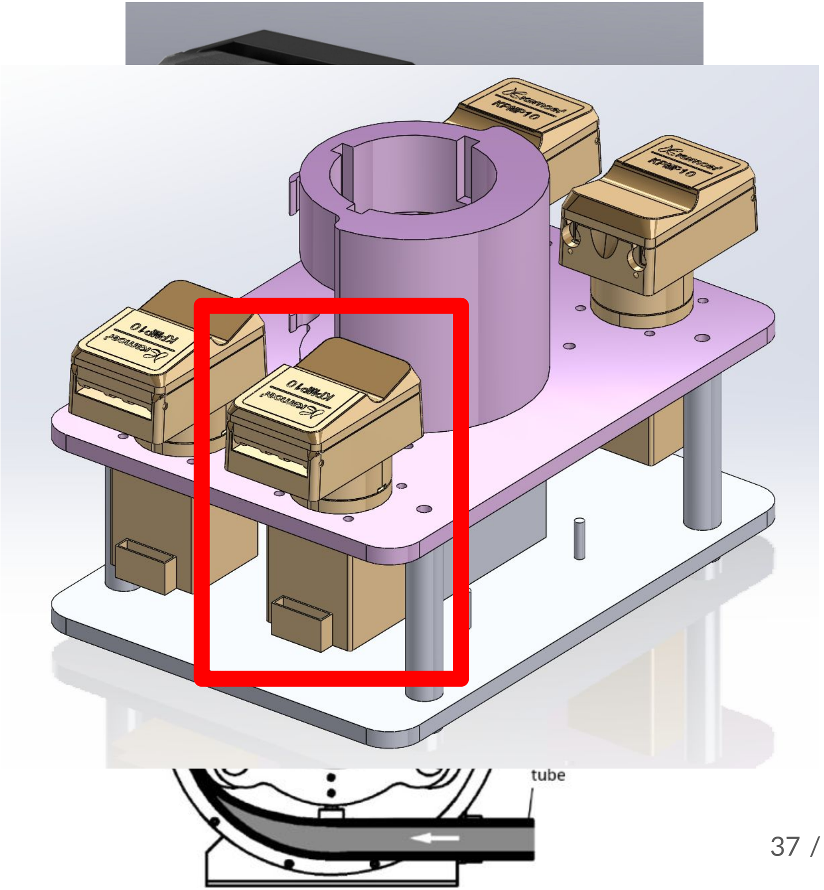
Pump Selection

Kamoer KPMP10 Peristaltic Pump

Requirement	Metric
Temperature control	25°C-50°C ± 1°C
Size - Benchtop scale	100 mL
Continuous Feed (Soft Req.)	0.1 - 5 mL/min
Run time	2 weeks

- 1 Inflow, 1 Outflow, 2 pH Control (Acid/Base Input)
- Pump Flow Rates: 0.1-5.9 mL/min
- MED55 PVC Tubing 1.6 x 3.2 mm ID x OD



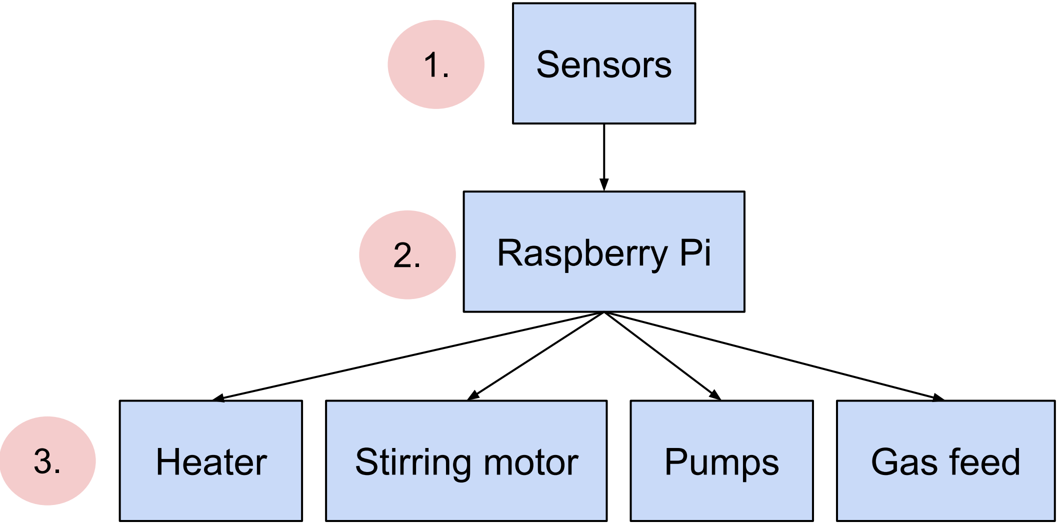


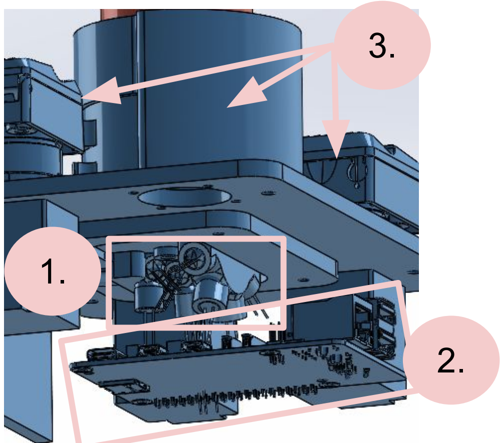
Software and Electronics

Controls Overview

- Required environment regulation
  - Temperature
  - pH
  - Dissolved Oxygen
  - Flow rate

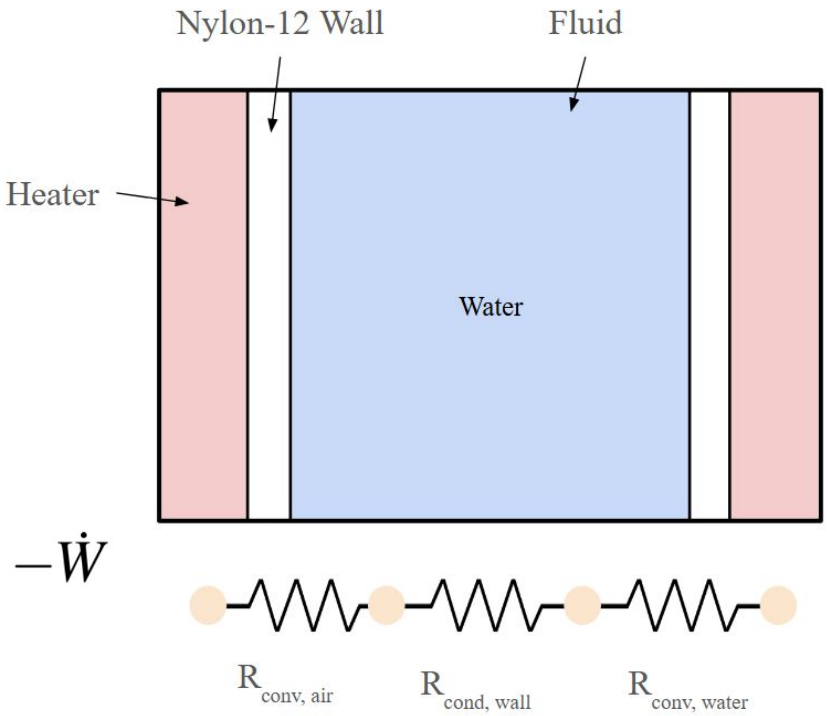
Requirement	Metric
Temperature control	25°C-50°C ± 1°C
Optical density sensing	0-2 ± 0.1au
Dissolved oxygen sensing	2-10 mg/L
pH sensing	4-9
Continuous Feed (Soft Req.)	0.1 - 5 mL/min





Temperature Control

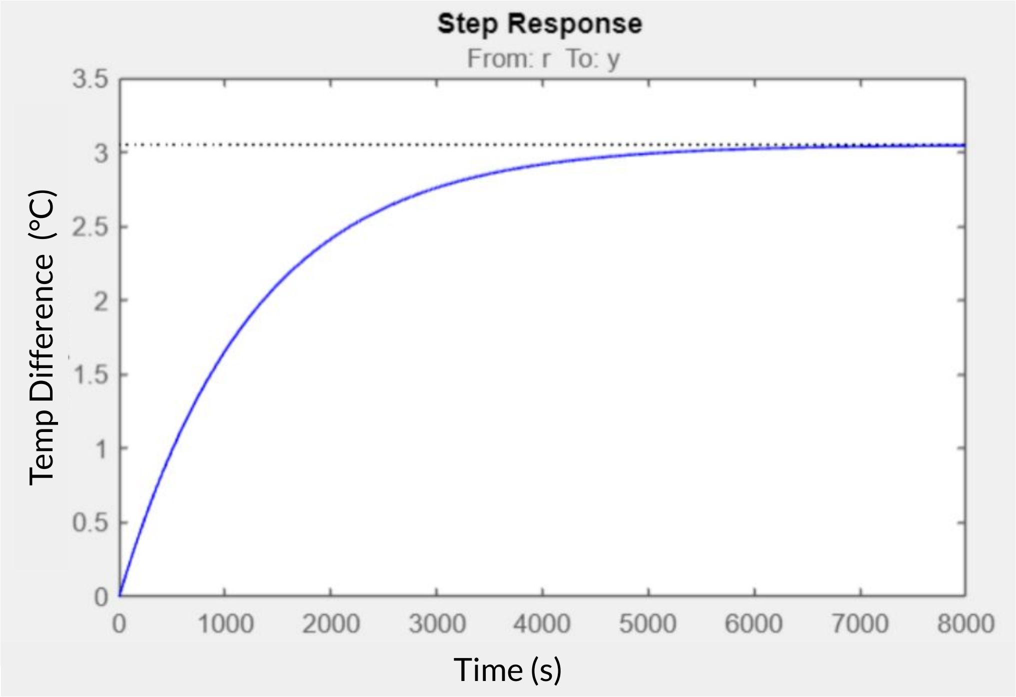
- Thermal model to understand dynamics
  - Governing equation from 1st law
  - Neglecting air convection
    - Vessel contacts heating pad



$$m_{water} * C_{p,water} * \frac{dT}{dt} - \frac{1}{R_{eff}} * T(t) = -\dot{W}$$

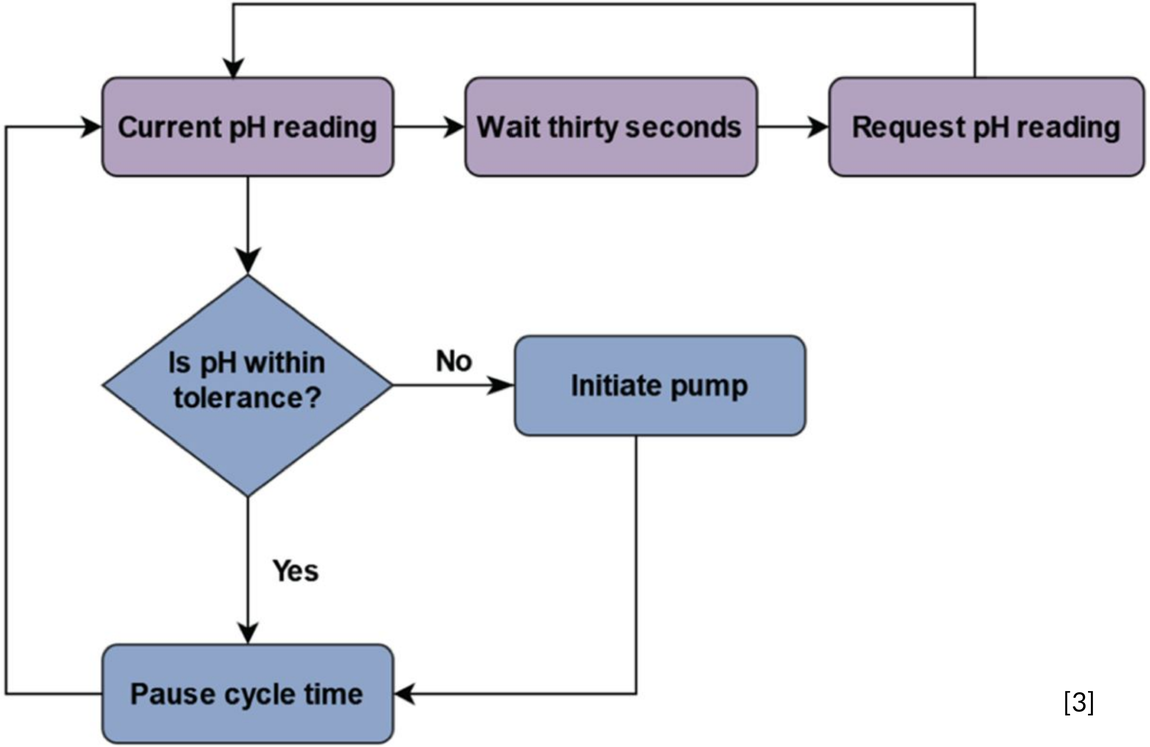
Temperature Control

- Open loop transfer function
  - First-order response
- Compensator design
  - Proportional-Integral control
  - Achieve heating time and no steady state error
- Input power step response graph
  - Time constant = 20 min
- Temp control scheme
  - High initial power
  - Decrease power until equilibrium



pH Control

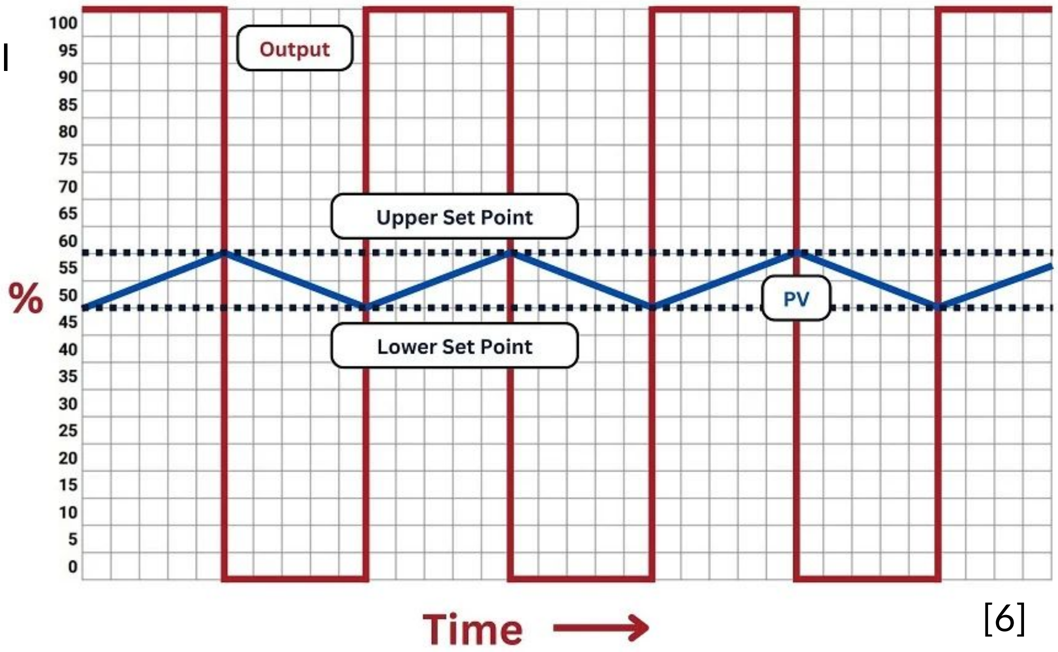
- Closed-loop feedback on-off controller with a deadband
- Acid/base addition
  - Acid: HCl, pH 0-1
  - Base: NaOH, pH 13-14
- User inputs
  - Target pH
  - Tolerance
  - Cycle time
- Small fixed amount of acid/base added by commanding pump on/off quickly



[3]

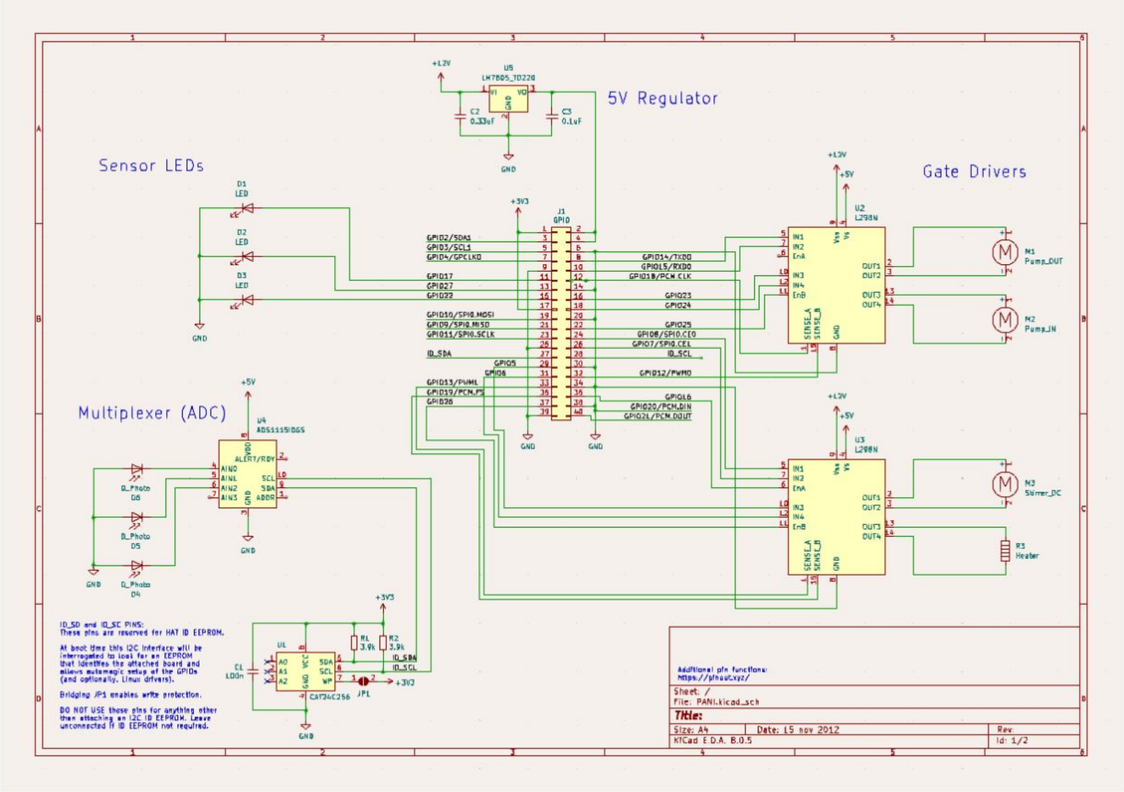
Dissolved Oxygen Control

- Closed-loop feedback on-off controller
  - Constants vary from vessel to vessel
  - Characterization testing required
- Gas addition
  - Oxygen O_2
  - Nitrogen N_2
  - Carbon Dioxide CO_2
- User inputs
  - Dissolved oxygen quantity
- Small fixed amount of gas added
  - Measure then add more if needed



[6]

Control Circuitry



Circuit Function Summary

120V AC input from wall

5V DC Regulated output

12V DC Regulated output

PWM Actuator control

Analog optical sensing with signal filtering

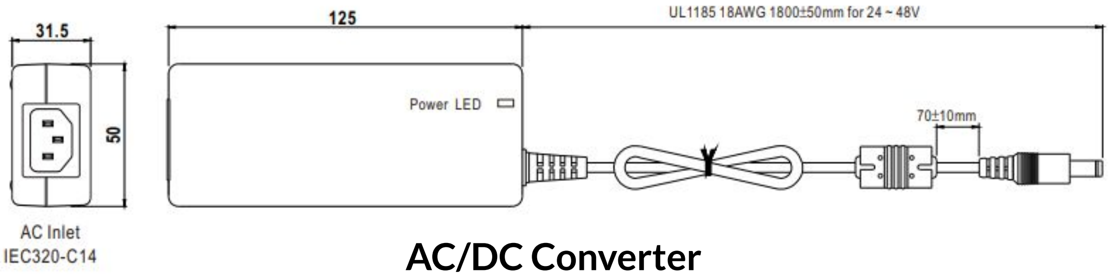
Power Electronics

Requirements:

120V AC → 12V DC
12V DC → 5V DC
PWM Control (25W peak)
Analog Signal Input

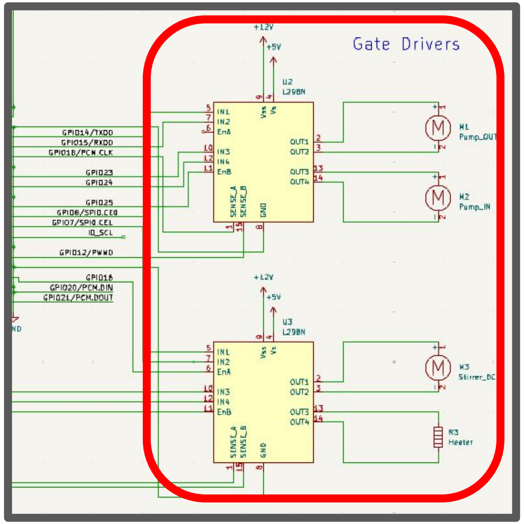
Hardware Selection:

12V Wall Adapter
LM7805 Voltage Regulator
L298N Gate Driver
ADS1115 Multiplexer



AC/DC Converter
P/N: GST60A12-P1J

12V Component	Steady Power Consumption
Pumps	0.5 W
Heaters	11 W
Stirring	1 W



Power Electronics

Requirements:

120V AC → 12V DC
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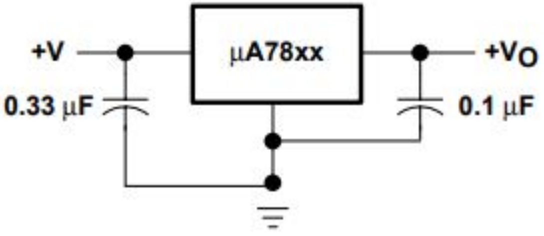
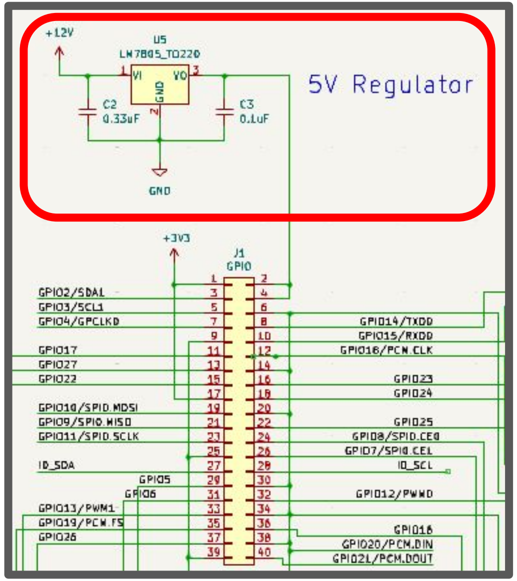


5V Voltage Regulator Circuit
P/N: LM7805

Components Utilizing 5V DC Source
Raspberry Pi VCC
Analog Input Multiplexer
Gate Driver Logic



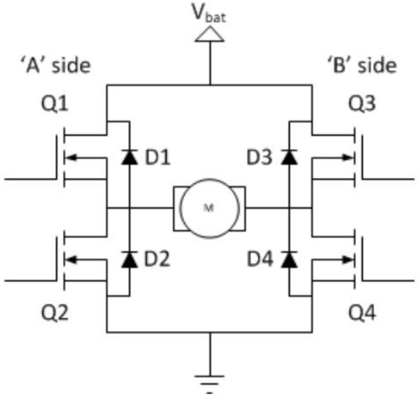
Power Electronics

Requirements:

120V AC → 12V DC
12V DC → 5V DC
PWM Control (25W peak)
Analog Signal Input

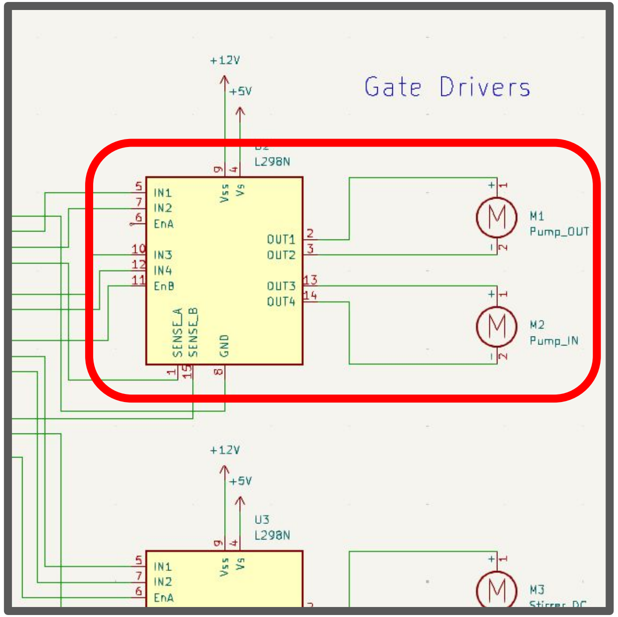
Hardware Selection:

12V Wall Adapter
LM7805 Voltage Regulator
L298N Gate Driver
ADS1115 Multiplexer



H-Bridge Working Principle Diagram
P/N: L298N

Components Utilizing H-Bridge PWM Control
Pump Motors
Heater
Stirring Motor



Analog Input

Requirements:

120V AC → 12V DC
12V DC → 5V DC
PWM Control (25W peak)
Analog Signal Input

Hardware Selection:

12V Wall Adapter
LM7805 Voltage Regulator
L298N Gate Driver
MCP3008 Multiplexer

8x Sensors

DO photodiode

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Temperature Sensor

7x Amplifier Circuits

Amplifier Circuit (Slide 19)

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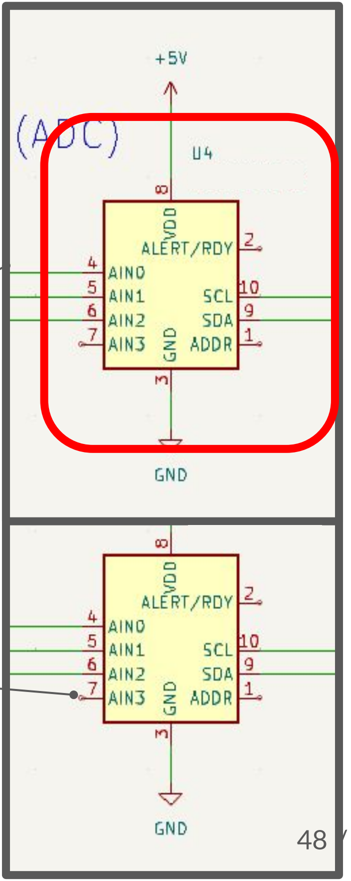
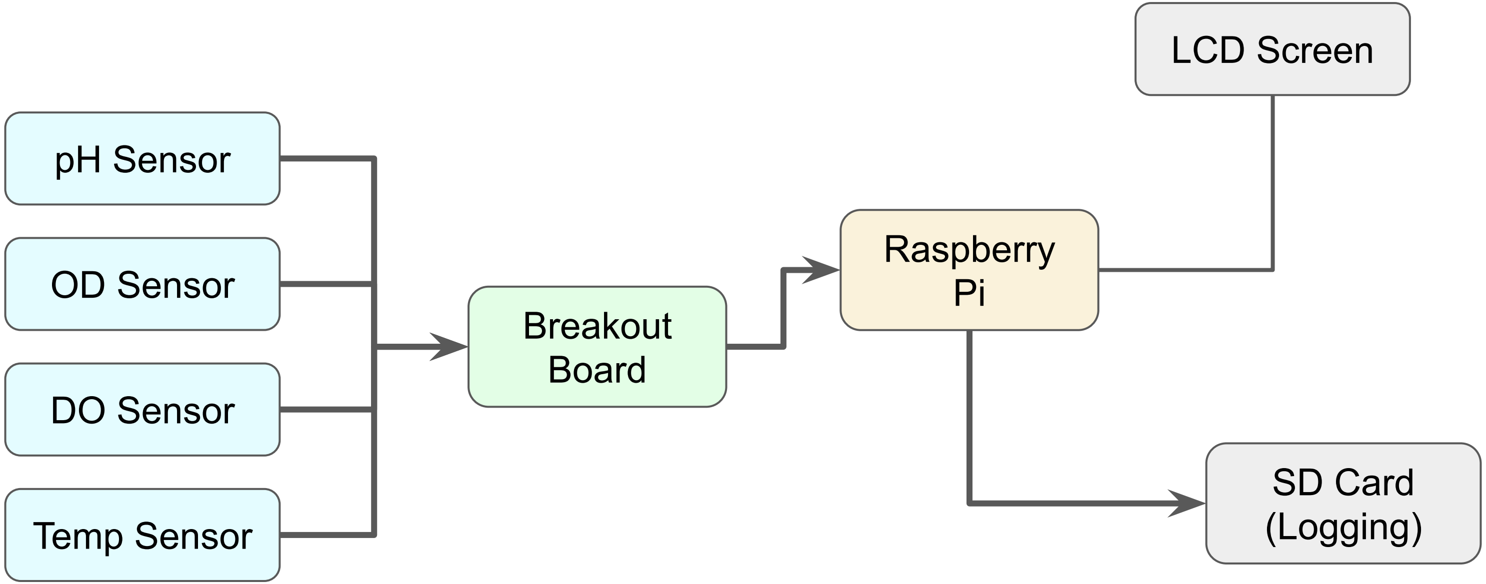


Data Bus


LCD Screen
pH Sensor
OD Sensor
DO Sensor
Temp Sensor
Breakout Board
Raspberry Pi
SD Card (Logging)

Project Implementation and Risk Management

Design Failure Mode and Effects Analysis (DFMEA)

Likelihood	**Concequence**				
	Insignificant	Minor	Moderate	Major	Catastrophic
Rare					2
Unlikely				1	
Moderate					
Likely					
Near Certain		3			

1 Sensor Calibration Failure

<u>Solution:</u> Have an auto-calibration feature

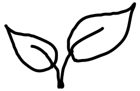
2 Faulty sealing or material compatibility

<u>Solution:</u> Ensuring proper sealing and material

3 Foaming

<u>Solution:</u> Anti-foam agent (added by user as necessary)

Lifecycle



Disposable

Run time requirement: 2 weeks/300 hours

Failure modes:

- Material degradation in acidic/basic conditions
- Stirring assembly wear

Reusable

Limiting Component: Motor lifetime

Pump motor lifetime: **6,000 hours** [4]
Stirring motor lifetime: 10,000 hours

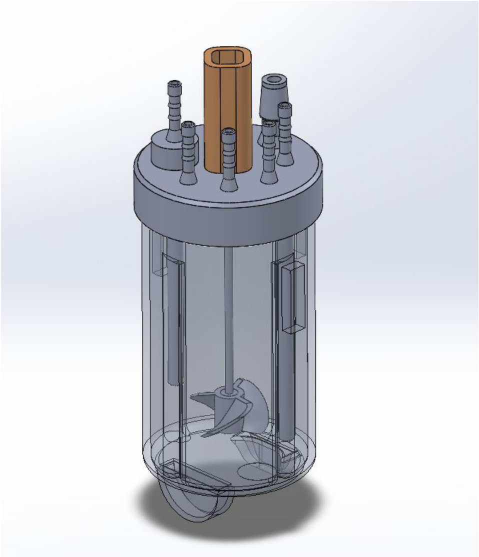
Usage assumptions:

- Pumps running continuously
- Room temperature usage
- No extreme forces are being applied
- Assume perfect models for electrical components

Overall Budget Breakdown

Category	Cost
Electronics and Sensors	$143.17
Heating and Fluids	$557.01
Mechanical and Structural Components	$57.00

Total Bioreactor Production Cost:	**$757.18**



Approximate Vessel (disposable) Cost	**$58.00**

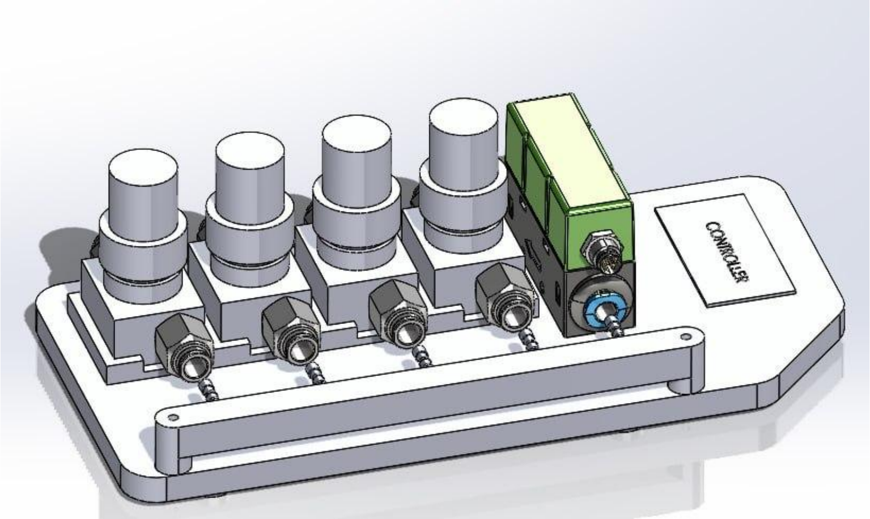
Next Steps and Potential Innovation

Next Steps:

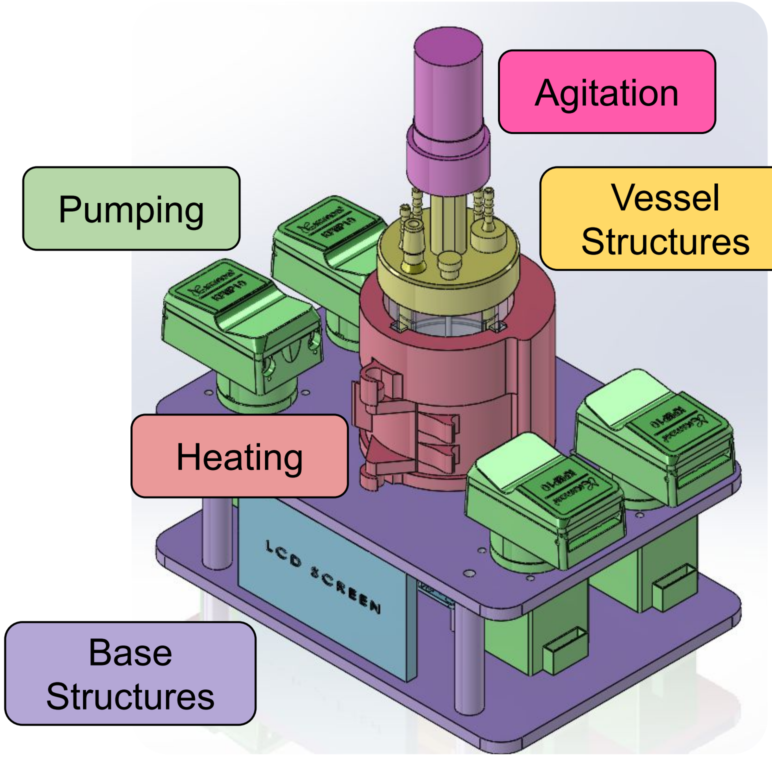
- Develop solution to foaming
- Gas box add-on

Potential Innovation:

- 4 in 1 non-invasive sensor package
  - Temperature
  - Dissolved Oxygen
  - Optical Density
  - pH

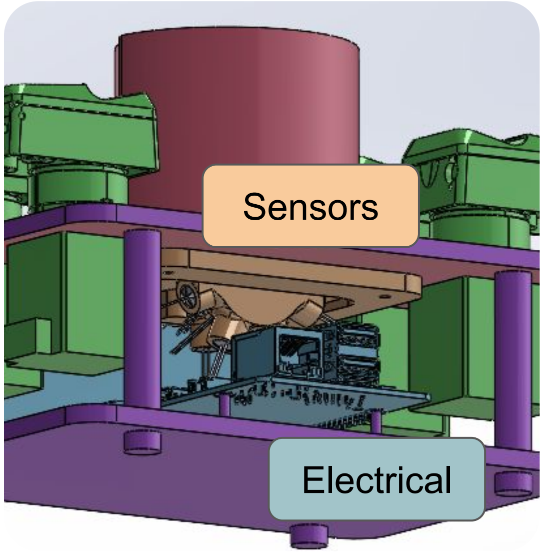


System Recap



Assembled Bioreactor

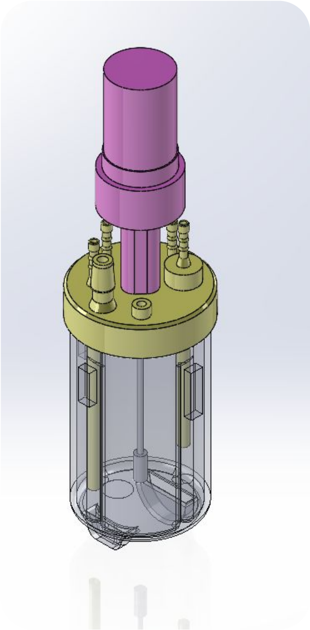
Reusable



Bioreactor Base

+

Disposable



Reactor Vessel + Stirring Motor

Appendix

BOM: Sensing & Electronics

#	Catagory	Component Name	Quantity	Cost per	Total Cost
1	pH Sensing	LED	1		$0.00
2	pH Sensing	Photodiode	2		$0.00
3	OD Sensing	LED	1	$0.67	$0.67
4	OD Sensing	Photodiode	2	$0.95	$1.90
5	OD Sensing	Reference Photodiode	1	$0.60	$0.60
6	DO Sensing	LED	1		$0.00
7	DO Sensing	Filter	1		$0.00
8	DO Sensing	Photodiode	2		$0.00
9	Power Electronics	Wall Power Adapter			$0.00
10	Temp Sensing	Thermistor			$0.00
11	Logic components	Raspberry Pi 5	1	$60.00	$60.00
12	Logic components	Raspberry Pi Touch Screen	1	$60.00	$60.00
13	Logic components	Sensor Routing PCB	1	$20.00	$20.00
		Total Cost:	$143.17		

BOM: Heating & Fluids

#	Catagory	Component Name	Quantity	Cost per	Total Cost
1	Agitation	Impeller	1	$1.00	$1.00
2	Heating	Heating Pad	1	$71.11	$71.11
3	Heating	TIM (1-2mm thick, one side sticky)	0.009	$104.00	$0.90
4	Motors/Pumps	Feed Inlet	1	$121.00	$121.00
5	Motors/Pumps	Main Output	1	$121.00	$121.00
6	Motors/Pumps	Acid Input	1	$121.00	$121.00
7	Motors/Pumps	Base Input	1	$121.00	$121.00
8	Motors/Pumps	Stirring motor	1		$0.00
		Total Cost:	$557.01		

BOM: Structures

#	Catagory	Component Name	Quantity	Cost per	Total Cost
1	Vessel	Membrane	1	$22.00	$22.00
2	Bioreactor Structure	Check Valve	1	$10.00	$10.00
3	Bioreactor Structure	Tubing	1	$15.00	$15.00
4	Bioreactor Structure	Bolts n shit	1	$10.00	$10.00
		Total Cost:	$57.00		

DO BOM

Electronics ->

Ru-Sensing layer

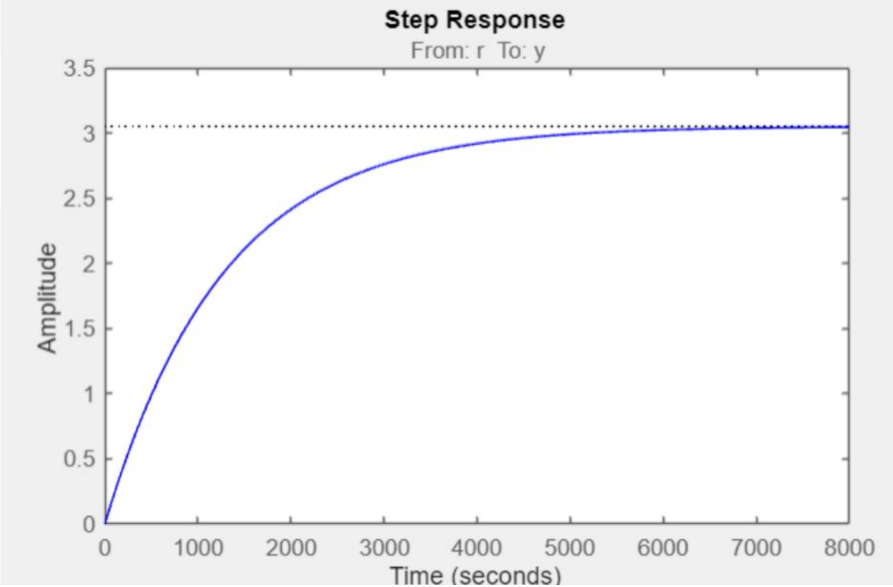
Part	Amount	Supplier	Cost
TEOS Precursor	10's mL	Sigma-Aldrich	$70/L
Ethanol	10's mL	TBD	small
DI water	10's mL	TBD	small
ruthenium(II) dichloride complex	1's mg	Medchem Express	$118/gram

Part	Specification	Supplier	Cost
LED	620 nm	Thorlabs	$7
Photodiode	350-1100 nm	Thorlabs	$17
Edge Filter	640 nm Longpass filter	Hoya	$32
Reference PD	SFH 213 FA	Mouser	$0.60
2x Resistor 1	500 ohms	TBD	small
2x Capacitor 1	1nF	TBD	small
2x Opamp	OP07	TI	$1.20

Control Transfer Function

$$H(s) = \frac{T(s)}{W(s)} = \frac{1}{m_{water} * C_{p,water} * s - \frac{1}{R_{eff}}}$$

$$m_{water} * C_{p,water} * \frac{dT}{dt} - \frac{1}{R_{eff}} * T(t) = -\dot{W}$$



Pump Lifetime

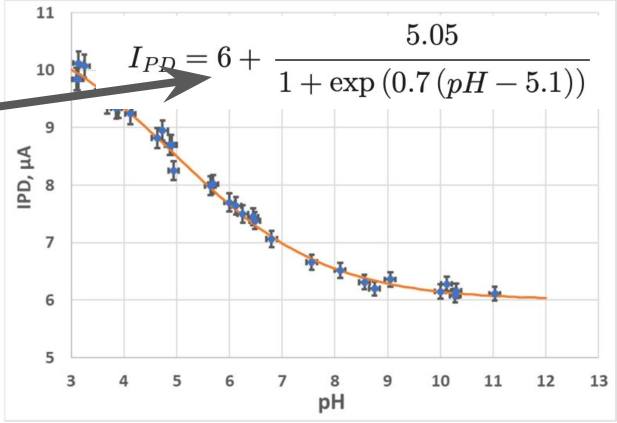
Technical Parameters

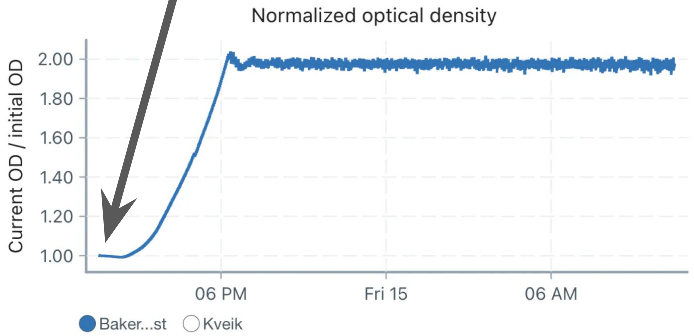
KPMP10-ST

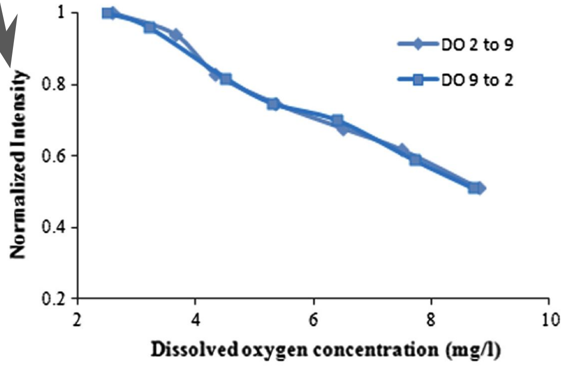
Motor Type	Stepper motor
Flow Rate Range	0.1-5.9ml/min
Power	10 W
Control Mode	Stepper motor driver
Weight	About 145 g
Rated Voltage	24V
Tube Lifetime	Depending on actual working conditions and media
Motor Lifetime	6000 h

Sensor Calibration Procedure

1. Remove new vessel from packaging
2. Fill new vessel with 10 mL of known pH
3. pH calibrates offset current
4. Rinse well and add 10mL of DI water
5. Leave lid partially covered for 10 minutes
6. Calculate equilibrium_oxygen(temp, altitude)
7. DO calibrates normalized intensity to equilibrium_oxygen
8. Pour out DI water
9. Fill vessel with starting contents for reaction
10. OD calibrates starting value







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[1]
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[2] McEvoy, Aisling K. et al., Dissolved Oxygen Sensor Based on Fluorescence Quenching of Oxygen-sensitive Ruthenium Complexes Immobilized in Sol-Gel derived Porous Silica Coatings

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