Progress Report for the Autumn Semester 2020-21

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Media Optimisation for both cellulase and betaglucosidase were done separately using sorghum leaf and pine needles as the substrate

Table1:Details of optimized media of cellulase and betaglucosidase

Substrate	**Pine needles(grinded and passed through a sieve of 0.850mm size and stored in a bags) 5gms substrate were taken in 250ml flask for betaglucosidase production** **Sorghum leaft and cotton seed oil cake(3:1)**
Organism	***Aspergillus niger* MTCC 7956** ***Trichoderma reesei* MTCC*7846***
Mode of fermentation	**Solid state fermentation**
Spore age	**8-10 days**
Spore size	**10^ 6 spores/ml**
Inoculum percentage	**15%**
Temp	**33.5**
pH	**5**
Moisture	**1:2**

Moisture media	**(Ammonium sulphate 2.4gm,CaCl2: 0.12 gm KH2PO4 :0.3gm,MgSO4: 0.055gm,tra 11mg and dissolved in 100ml to make moisture media).Molasses and Quinoa(8%) the carbon source in the solid substrate**
No of days of fermentation	**5**

The maximum cellulase activity was obtained as 18FPU/gds and betaglucosidase as 5.99IU/gds.The crude cellulase was further ultrafiltered to obtain a activity of 66FPU/gds

The kinetic and transport parameters were estimated as follows:

1.Particle density

A known mass of biomass is being flooded with known volume of water in a known volume of measuring cylinder or beaker. The substrate particle density can then be calculated as:

$$\rho_S=\frac{(m_{total}-m_{container})-m_w}{v\ \ total-\frac{m_w}{p_w}}=\frac{m_s}{v_s}$$

Where , $m_{total}$is the mass of the system after flooding,

$m_{container}$ is the mass of the empty container

m_w is the mass of the water added to flood the bed

v_{total} =total volume of flooded bed(L)

ρ_w=density of water(g/L) and

m_s= mass of the substrate particle(g)

2. Bed packing density

It is determined by packing the substrate, prepared in a manner identical to preparation for the fermentation, to fill a container of known volume(V liters) and mass and reweighing. The difference between the masses of the container when it is packed with substrate and hen it is empty is the packed mass of the bed(g).The packing density is calculated as:

$$\rho_b=\frac{m_p}{V}$$

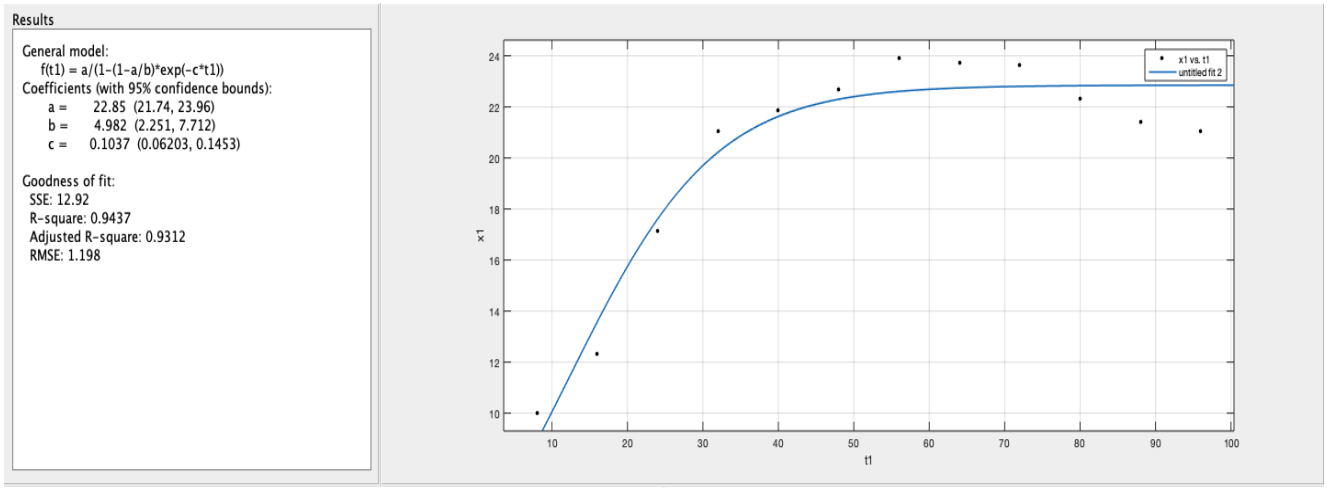
3. Porosity

The way that the substrate bed as a whole packs is important in determining the effectiveness of aeration. A correlation has been developed to find out the porosity or void fraction from substrate density and bed packing density.

$$\in= 1-\frac{v_s}{v_b}$$

$$=1-\frac{\frac{m_s}{\rho_s}}{\frac{m_b}{p_b}}$$

$$=1-\frac{\rho_b}{\rho_s}$$



4.Substrate thermal conductivity

The substrate thermal conductivity was measured using the KS-1 probe of a thermal property analyser(KD2 Pro,USA) which was found to be 0.061W/m.K.

The values of density of air, heat capacity of air and latent heat of vaporization of water were obtained from standard literature.

5. Biomass estimation

Once enzyme extraction is done the fermented substrate is dried in oven and taken for acid hydrolysis. After 1day incubation the samples are taken and autoclaved foe one hour and neutralized. Glucosamine assay is performed to check the release of glucosamine from the dried fermented substrate.

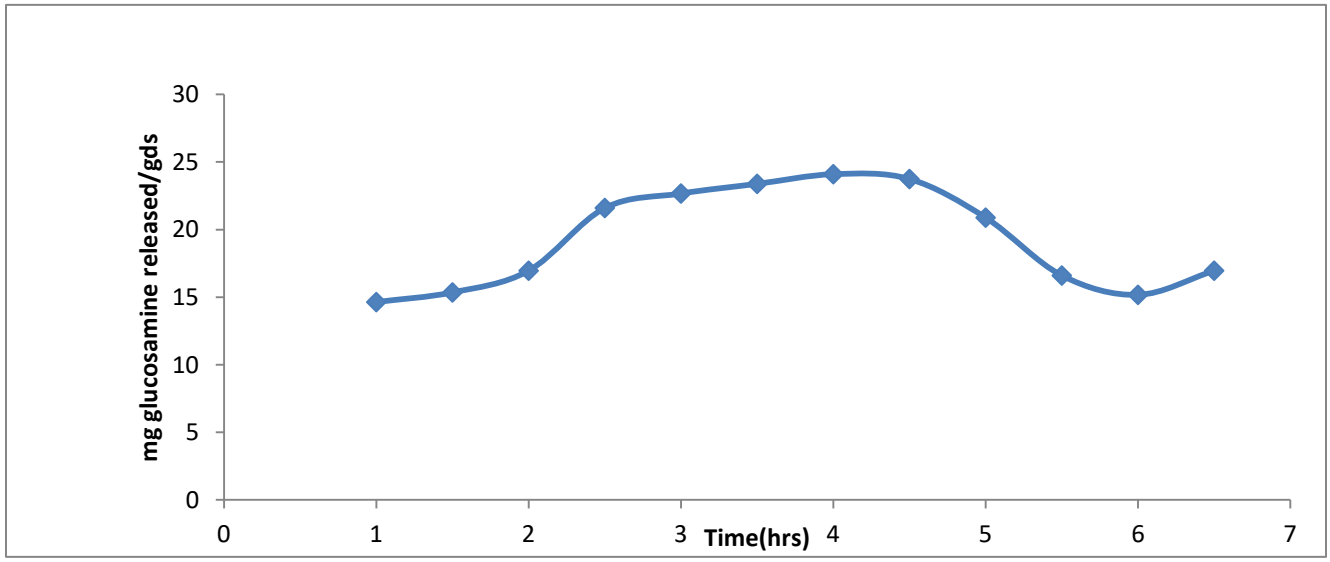


Fig1:Biomass Production by glucosamine assay

Table 2:Estimation of kinetic and transport parameters

S.no	Parameters	Values
1.	Substrate density (ρs)	1250kg/m3
2.	Bed packing density (ρb)	750kg/m3
3.	Void fraction(porosity)(ε)	0.4016
4.	Substrate thermal conductivity	0.061 W/m.K
5.	Initial biomass(X0)	5g
6.	Maximum biomass(Xm)	23.39mg glucosamine /gram dry substrate.
7.	Specific growth rate(μ)	0.1034 /hr
8.	Heat capacity of dry substrate(Cps)	4349.8 J/kg/Kelvin
9.	Heat capacity of dry air(Cpg)	1006J/kg/°C
10.	Heat capacity of water vapour(Cpv)	1880 J/kg/°C
11.	Heat capacity of liquid water(Cpw)	4184 J /kg/°C
12.	Enthalpy of vaporization of water(λ)	2.414 x 10^6 J kg/water
13.	Metabolic heat from growth(YQ)	8.366 x 10^6Jkg/substrate

14.	**Water activity**	**0.9**

Designing and Specification of newly fabricated SSF Bioreactor

- Height of the module= 6″
- Diameter of the module= 12″ (Outer) and 10.5″ (inner)
- Material of construction: SS 316
- Upper chamber height= 4.5″
- Lower chamber height= 1.5″
- Pore size in separating plate= 2mm
- Rubber fitting(O- rings) in probe ports to ensure air tight.
- Silicon Sealing to ensure proper air tight conditions in the lid
- Closing the lid with handle like of autoclave which can be hand tightened
- Viewing glass in the reactor=3*3 “ to be placed at a height of 4cm from the upper chamber.
- Area between pores= 1 cm $\times$ 1 cm
- Diameter of the probe= 5 mm
- Total number of probes on upper chamber= 5 (one on upper end and four on lower end)placed at equal interval of 1cm.(four temperature sensors at a height of 2cm 4cm and 6cm and 10cm) and one moisture sensor at 4cm height
- Inlet air hole= Location: Lower chamber, Size: 5mm
- Outlet air hole= Location: Upper chamber, Size= 5mm

- Nozzle= hole diameter: 1 mm
- Thickness of the module sheet: 0.5 cm
- Mixing Blades: Attached to the shaft in a rotating pattern
- One digital rpm controller attached to the motor(mixing blades) for intermittent mixing.
- Cooling coils were made in a circular pattern and which is attached to the head plate.
- All the pipings should be placed through punching system
- Pressure guage to be placed over the reactor top.
- Blades connected to central shaft via gear system. Diameter mixing shaft= inner (3.1 cm) and outer 3.8 cm.
- Silicon rubber for each to seal each module and to ensure no leakage of air.
- **Note:** The order is for one such stacks arranged vertically around central shaft.If it works successfully we will order two more modules as such. It shall be responsibility of the fabricator to ensure proper mixing in the reactor through alignment of motor and reactor mechanical parts (through gear system). The motor and gear box shall be supplied by me as required by the fabricator.

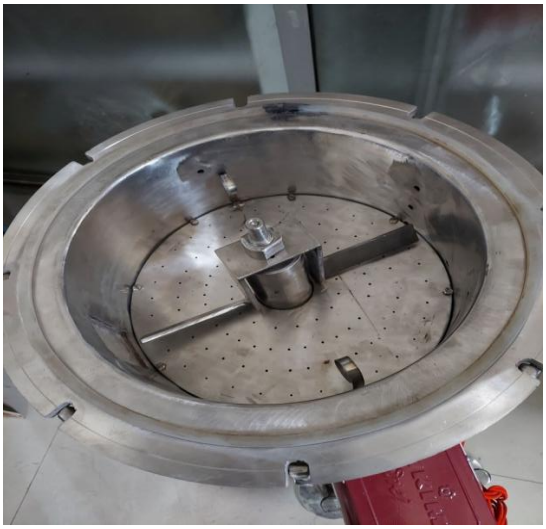
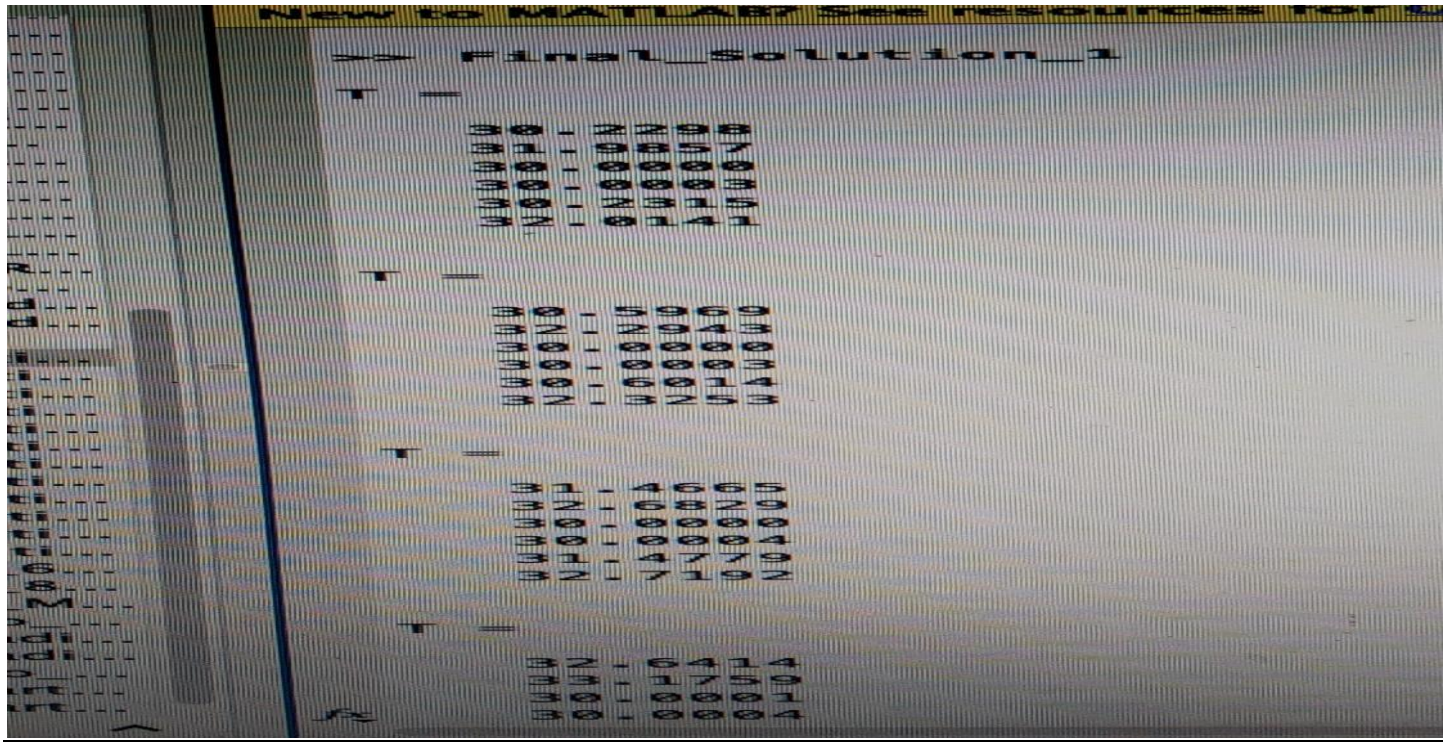
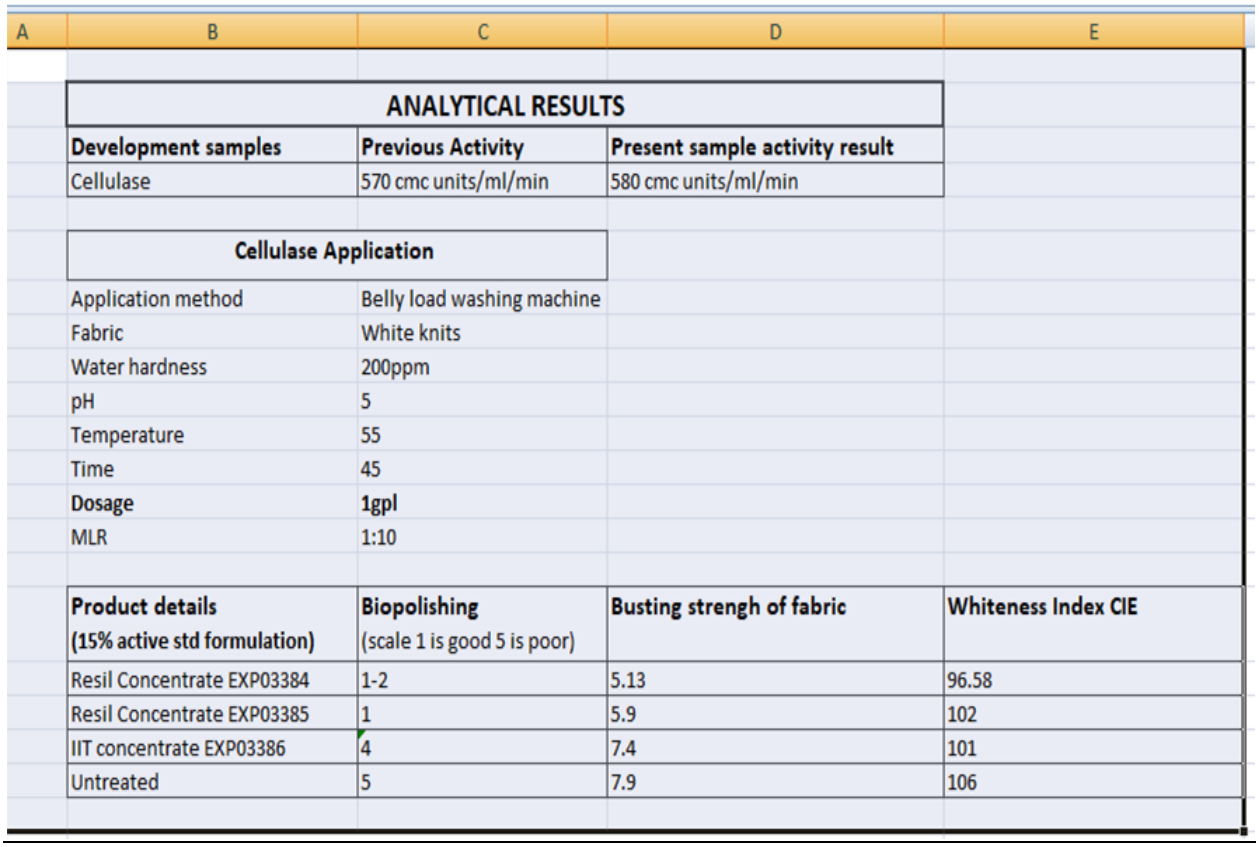


Fig 2a: Design of the SSF Bioreactor,2c:Mixing blades over the perforated plate,2b:Cooling coils

Matlab simulation



The different bed temperatures were estimated numerically in matlab simulation which are presently performed experimentally to validate the results



ANALYTICAL RESULTS			
Development samples	**Previous Activity**	**Present sample activity result**	
Cellulase	570 cmc units/ml/min	580 cmc units/ml/min	
Cellulase Application			
Application method	Belly load washing machine		
Fabric	White knits		
Water hardness	200ppm		
pH	5		
Temperature	55		
Time	45		
Dosage	**1gpl**		
MLR	1:10		
Product details (15% active std formulation)	**Biopolishing** (scale 1 is good 5 is poor)	**Busting strengh of fabric**	**Whiteness Index CIE**
Resil Concentrate EXP03384	1-2	5.13	96.58
Resil Concentrate EXP03385	1	5.9	102
IIT concentrate EXP03386	4	7.4	101
Untreated	5	7.9	106

Fig 3:Validation report of cellulase for Biopolishing from Resil Chemical Pvt Ltd

Dosage	Glucose	xylose	glu rel(mg/ml)	xylose rel(mg/ml)
control 30	3324517	8993174	18.28617931	52.23153811
control 60	3873102	8993174	21.30361723	52.23153811
control 90	3589536	8468694	19.74389029	49.18540589
control 120	5020231	11781737	27.61328765	68.42725884
0.1% cellulase 30	5238930	11221799	28.81622002	65.17518977
0.1% cellulase 60	4592904	10753891	25.26281745	62.45762259
0.1% cellulase 90	2918333	16829681	16.0520041	97.74525929
0.1% cellulase 120	4467954	10440558	24.57554224	60.63781297

0.5% cellulase 30	4525891	10389125	24.89421902	60.33909478
0.5% cellulase 60	3702464	8515324	20.36503967	49.45622869
0.5% cellulase 90	4142260	9882656	22.78409438	57.39756881
0.5% cellulase 120	3826258	9438592	21.04595641	54.81848541
1% cellulase 30	6088022	14682322	33.48656719	85.27359318
1% cellulase 60	5873916	14488502	32.30889817	84.14790422
1% cellulase 90	5812362	14137823	31.97032643	82.11119242
1% cellulase 120	5679862	13662753	31.24152319	79.35202899

Table 4: Effect of Cellulase in sugar release in apple juice

	%Transmittance@660nm
Distilled water	98.668
Unpasteurized fruit juice	71.891
Pasteurized fruit juice	64.496
Different dosage of enzyme with varying time	
Dosage	Transmittance@660nm
0.1%(30min)	81.499
0.1%(60min)	83.208
0.1% (90min)	85.73
0.1%(120min)	84.443

0.5%(30min)	81.915
0.5%(60min)	82.257
0.5%(90min)	85.211
0.5%(120min)	86.036
1%(30min)	81.915
1%(60min)	85.766
1%(90min)	85.654
1%(120min)	84.1

Table 5:Effect of cellulase in checking transmittance at different dosages

Sample	Viscosity(Pa.s)
Fruit juice pasteurized	0.27185
Control,30min	0.002412
Control,60min	0.002214
Control,90min	0.002201
Control,120min	0.002274
0.1%,30min	0.002031
0.1%60min	0.004331

0.1%,90min	0.002154
0.1%,120min	0.002209
0.5%,30min	0.002098
0.5%, 60min	0.002116
0.5%,90min	0.001985
0.5%,120min	0.02209
1%,30min	0.02051
1%, 60min	0.002074
1%,90min	0.001992
1%,120min	0.01981

Table 6:Effect of cellulase in viscosity reduction in apple fruit juice

Sample	Turbidity (NTU)
Control unpasteurized juice	453
Pasteurized juice	449
0.1% ,30min	439
0.1%,60min	417
0.1%,90min	407

0.1%,120min	**375**
0.5% ,30min	**412**
0.5%,60min	**406**
0.5%,90min	**376**
0.5%,120min	**386**
1%,30min	**413**
1%,60min	**386**
1%,90min	**369**
1%,120min	**348**

Table 7:Effect of cellulase in turbidity reduction of apple fruit juice

<u>Workplan for present semester</u>

Characterization of the enzyme

Optimisation of Air flow rate,temperature and humidity in the bioreactor

Optimisation of mixing events(Frequency ,duration and intensity)