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## APPENDIX A

### Streaking Plates

In microbiology, **Streaking** is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested. The streaking is done using a sterile tool, such as a cotton swab or commonly an inoculation loop. This is dipped in inoculums such as a broth or patient specimen containing many species of bacteria.

The sample is spread across one quadrant of a Petri dish containing a growth medium, usually an agar plate which has been sterilized in an autoclave. Choice of which growth medium is used depends on which microorganism is being cultured, or selected for. Growth media are usually forms of agar, a gelatinous substance derived from seaweed.



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The quadrant which is first inoculated will contain too many bacteria to select one colony. By re-sterilizing the loop and dragged it across the previously inoculated quadrant, only some of the original sample is introduced to new sections of the plate. The loop is re-sterilized and a new quadrant inoculated in the same manner. Each time the loop gathers fewer and fewer bacteria until it gathers just single bacterial cells that can grow into a colony.

Dependant on the strain, the plate may then be incubated, usually for 24 to 36 hours, to allow the bacteria to reproduce. At the end of incubation there should be enough bacteria to form visible colonies in the areas touched by the inoculation loop. From these mixed colonies, single bacterial or fungal species can be identified based on their morphological (size/shape/colour) differences, and then sub-cultured to a new media plate to yield a pure culture for further analysis.

Automated equipments are used at industrial level for streak plating the solid media in order to achieve better sterilization and consistency of streaking and for reliably faster

work. While streaking manually it is important to avoid scratching the solid medium as subsequent streak lines will be damaged and non-uniform deposition of inoculum at damaged sites on the medium yield clustered growth of microbes which may extend into nearby streak lines.



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## APPENDIX B

### Gram Staining

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process. Gram-positive cells may become gram negative through mechanical damage, conversion to protoplasts, or aging, in which autolytic enzymes attack the walls.

### Reagents:

- Crystal violet (primary stain)
- Iodine solution/Gram's Iodine
- Decolorizer (e.g. ethanol)
- Safranin (secondary stain)
- Water (preferably in a squirt bottle)

### Technique:

1. Make a slide of cell sample to be stained. Heat fix the sample to the slide by carefully passing the slide with a drop or small piece of sample on it through a Bunsen burner three times.
2. Add the primary stain (crystal violet) to the sample/slide and incubate for 1 minute. Rinse slide with a gentle stream of water for a maximum of 5 seconds to remove unbound crystal violet.
3. Add Gram's iodine for 1 minute- this is a mordant, or an agent that fixes the crystal violet to the bacterial cell wall.
4. Rinse sample/slide with acetone or alcohol for 3 seconds and rinse with a gentle stream of water. The alcohol will decolorize the sample if it is Gram negative, removing the crystal violet. However, if the alcohol remains on the sample for too long, it may also decolorize Gram positive cells.
5. Add the secondary stain, safranin, to the slide and incubate for 1 minute. Wash with a gentle stream of water for a maximum of 5 seconds. If the bacteria is Gram positive, it will retain the primary stain (crystal violet) and not take the secondary stain (safranin), causing it to look violet/purple under a microscope. If the bacteria are Gram negative, it will lose the primary stain and take the secondary stain, causing it to appear red when viewed under a microscope.

## APPENDIX C

### Preparation of Blood Agar

Composition of blood agar base as follows.

Ingredient	Amount g/l
Protease peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0

### Method

39.5 g of blood agar was accurately measured and transferred in to an autoclavable container. Then 1l of distilled water was added to it and shaken well. This mixture was autoclaved at 121 °C for 15 minutes. When it was cool about to 50 °C- 60 °C 5-10 % blood was added to the agar mixture and shaken slowly. (For this process, human blood is kindly supplied by the blood bank or sheep blood is supplied by MRI). After the mixing properly, it was poured to sterilized petri dishes under sterile conditions. Plates were kept for 5-10 minutes for solidification. Then they were kept at 37 °C incubator to evaporate excess water vapor before usage.

## APPENDIX D

### Carbon Source Optimization for BC production by AX

#### 1. Average wet weight and wet yield fraction of BC with respect to control sample in different media at different pH values

Initial pH	Average wet weight /g (W1)						Wet yield fraction of BC with respect to control sample				
	Glucose	Fructose	Sucrose	Mannitol	Trehalose	Control sample	Glucose	Fructose	Sucrose	Mannitol	Trehalose
3.5	0.3064	0.0418	0.0000	0.0863	0.3792	0.0000	-	-	-	-	-
4.0	0.7073	0.5774	0.0415	0.7490	0.7700	0.2328	- 2.0382	- 1.4802	0.8217	- 2.2173	- 2.3075
4.5	<b>0.7552</b>	0.4122	0.4095	0.9741	0.4944	0.8308	0.0909	0.5038	0.5071	- 0.1724	0.4049
5.0	0.7278	0.3844	0.4908	0.5021	0.665	0.6483	- 0.1226	0.4071	0.2429	0.2256	- 0.0257

#### 2. Average dry weight and dry yield fraction of BC with respect to control sample in different media at different pH values

Initial pH	Average Dry weight /g (W2)						Dry yield fraction of BC with respect to control sample				
	Glucose	Fructose	Sucrose	Mannitol	Trehalose	Control sample	Glucose	Fructose	Sucrose	Mannitol	Trehalose
3.5	0.0167	0.0082	0.0000	0.0080	0.0184	0.0000	-	-	-	-	-
4.0	0.0365	0.0388	0.0061	0.0285	0.0368	0.0131	- 1.7862	- 1.9618	0.5343	- 1.1755	- 1.8092
4.5	0.0462	0.0185	0.0235	<b>0.0632</b>	0.0308	0.0536	0.1380	0.6548	0.5616	- 0.1791	0.4253
5.0	<b>0.0824</b>	<b>0.0805</b>	<b>0.1020</b>	0.0275	<b>0.1165</b>	<b>0.1531</b>	0.4617	0.4742	0.3337	0.8204	0.23906

**3. Final pH of culture media and %pH reduction fraction with respect to the control sample after fermentation period**

Initial PH	Final pH						% pH reduction fraction					
	Glucose	Fructose	Sucrose	Mannitol	Trehalose	Control sample	Glucose	Fructose	Sucrose	Mannitol	Trehalose	Control sample
3.5	3.41	3.43	3.48	3.82	3.48	3.46	2.57	2.00	0.57	- 9.14	0.57	1.14
4.0	3.93	4.03	4.02	4.12	4.13	4.13	1.75	- 0.75	- 0.50	- 3.00	- 3.25	- 3.25
4.5	4.16	4.28	4.08	4.12	4.31	4.18	7.56	4.89	9.33	8.44	4.22	7.11
5.0	4.3	4.32	4.17	4.13	4.96	4.2	14.00	13.60	16.60	17.40	0.80	16.00



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## APPENDIX E

### Nitrogen Source Optimization for BC Production by AX

#### 1. Wet weight and wet yield fractions of BC in different media at different pH values

Initial pH	Average wet weight /g (W1)				Wet yield fraction with compared to control sample		
	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	CO(NH <sub>2</sub> ) <sub>2</sub>	Control sample	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	CO(NH <sub>2</sub> ) <sub>2</sub>
3.5	0.0090	0.1926	0.1613	2.4835	0.9964	0.9224	0.9351
4.0	1.9925	2.0784	1.4848	1.5471	- 0.2879	- 0.3434	0.0403
4.5	1.1841	1.5058	1.4483	1.0876	- 0.0887	- 0.3845	- 0.3316
5.0	1.0273	0.5272	1.4285	1.3275	0.2261	0.6029	- 0.0761

#### 2. Dry weight and dry yield fractions of BC in different media at different pH values

Initial pH	Average Dry weight /g (W2)				Dry yield fraction with compared to control sample		
	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	CO(NH <sub>2</sub> ) <sub>2</sub>	Control sample	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	CO(NH <sub>2</sub> ) <sub>2</sub>
3.5	0.0010	0.0076	0.0087	0.0735	0.9864	0.8966	0.8816
4.0	0.0789	0.0898	0.0453	0.0457	- 0.7265	- 0.9650	0.0088
4.5	0.0427	0.0783	0.0374	0.0342	- 0.2485	- 1.2895	- 0.0936
5.0	0.0327	0.0654	0.0352	0.0387	0.1550	- 0.6899	0.0904

#### 3. Final pH and % pH reduction fraction of different media after fermentation period

Initial PH	Final pH				% pH reduction fraction			
	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	CO(NH <sub>2</sub> ) <sub>2</sub>	Control sample	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	CO(NH <sub>2</sub> ) <sub>2</sub>	Control sample
3.5	3.41	3.45	3.46	3.47	2.57	1.43	1.14	0.86
4.0	3.41	3.42	3.59	3.61	14.75	14.50	10.25	9.75
4.5	3.42	3.55	3.62	3.77	24.00	21.11	19.56	16.22
5.0	3.45	3.59	3.74	3.81	31.00	28.20	25.20	23.80

## APPENDIX F

### Mechanical Properties of Each Moisture Content BC in two perpendicular orientations

0-5 % moisture content BC							
Graph No	Maximum force/N	Average Thickness/mm	Cross section area/ mm <sup>2</sup>	Stress $\times 10^6$ /Nm <sup>-2</sup>	Extension/mm	Strain	Y module/MPa
(A-B) 1	8.80	0.09	0.270	32.59	6.12	0.245	133.02
(A-B) 2	8.25	0.073	0.219	37.67	7.80	0.312	120.74
(A-B) 3	5.00	0.070	0.210	23.81	4.52	0.181	131.54
<b>Average</b>				<b>31.35</b>		<b>0.246</b>	<b>128.43</b>
(B-A) 4	7.20	0.060	0.180	-	2.28	-	-
(B-A) 5	10.00	0.060	0.180	55.55	2.42	0.097	572.68
(B-A) 6	11.00	0.073	0.219	50.23	2.07	0.083	605.18
<b>Average</b>				<b>52.89</b>		<b>0.090</b>	<b>588.93</b>

5-10 % moisture content BC							
Graph No	Maximum force/N	Average Thickness/mm	Cross section area/ mm <sup>2</sup>	Stress $\times 10^6$ /Nm <sup>-2</sup>	Extension/mm	Strain	Y module/MPa
(A-B) 1	10.00	0.24	0.720	13.89	8.20	0.328	42.34
(A-B) 2	8.75	0.23	0.690	12.68	7.43	0.297	42.69
(A-B) 3	10.00	0.21	0.630	15.87	8.41	0.336	47.23
<b>Average</b>				<b>14.14</b>		<b>0.320</b>	<b>44.08</b>
(B-A) 4	4.00	0.080	0.240	-	1.73	-	-
(B-A) 5	10.14	0.073	0.219	46.30	3.45	0.138	335.50
(B-A) 6	7.50	0.060	0.180	41.66	3.54	0.141	295.46
<b>Average</b>				<b>43.98</b>		<b>0.1395</b>	<b>315.48</b>

10-15 % moisture content BC							
Graph No	Maximum force/N	Average Thickness/mm	Cross section area/ mm <sup>2</sup>	Stress $\times 10^6$ /Nm <sup>-2</sup>	Extensio n/mm	Strain	Y module/ MPa
(A-B) 1	14.15	0.19	0.57	24.82	5.14	0.206	120.48
(A-B) 2	12.00	0.20	0.60	20.00	4.40	0.176	113.63
(A-B) 3	12.75	0.15	0.45	28.33	5.97	0.239	118.53
<b>Average</b>				<b>24.38</b>		<b>0.207</b>	<b>117.54</b>
(B-A) 4	8.35	0.26	0.78	10.70	7.58	0.303	35.31
(B-A) 5	8.00	0.30	0.90	8.89	8.41	0.336	26.46
(B-A) 6	10.50	0.33	0.99	10.60	8.24	0.330	32.12
<b>Average</b>				<b>30.19</b>		<b>0.323</b>	<b>31.29</b>

15-20 % moisture content BC							
Graph No	Maximum force/N	Average Thickness/mm	Cross section area/ mm <sup>2</sup>	Stress $\times 10^6$ /Nm <sup>-2</sup>	Extensio n/mm	Strain	Y module /MPa
(A-B) 1	10.50	0.20	0.60	17.50	5.85	0.234	74.78
(A-B) 2	12.50	0.16	0.48	26.04	7.03	0.281	92.66
(A-B) 3	16.65	0.28	0.85	19.47	7.09	0.283	68.79
<b>Average</b>				<b>21.0</b>		<b>0.266</b>	<b>78.74</b>
(B-A) 4	15.50	0.33	0.99	15.65	8.58	0.343	45.62
(B-A) 5	8.75	0.26	0.78	11.21	6.72	0.268	41.82
(B-A) 6	10.00	0.24	0.72	13.89	8.51	0.340	40.85
<b>Average</b>				<b>18.8</b>		<b>0.317</b>	<b>42.76</b>

20-25 % moisture content BC							
Graph No	Maximum force/N	Average Thickness/mm	Cross section area/ mm <sup>2</sup>	Stress $\times 10^6$ /Nm <sup>-2</sup>	Extension /mm	Strain	Y module/ MPa
(A-B) 1	10.5	0.34	1.02	10.29	8.76	0.350	29.40
(A-B) 2	12.0	0.33	0.99	12.12	9.02	0.360	33.66
(A-B) 3	9.2	0.29	0.87	10.57	8.32	0.333	31.74
<b>Average</b>				<b>10.99</b>		<b>0.347</b>	<b>31.60</b>
(B-A) 4	11.50	0.313	0.939	12.24	10.13	0.405	30.22
(B-A) 5	11.35	0.316	0.948	11.97	8.24	0.329	36.38
(B-A) 6	8.50	0.330	0.990	8.58	7.08	0.283	30.31
<b>Average</b>				<b>10.93</b>		<b>0.339</b>	<b>32.30</b>



### **3.% Saline reabsorption (Dry basis) of different moisture content BC samples**

pellicle no	wet weight	dry weight	% moisture	Range of moisture	weight of pellicles after immersing in 0.9% normal saline solution at different time intervals								%Reabsorbtion of saline (dry basis) at different timer intervals							
					2h	4h	6h	20h	24h	28h	48h	2h	4h	6h	20h	24h	28h	48h		
					1	0.5242	0.0104	1.98	2-3	0.076	0.0925	0.0971	0.1024	0.1028	0.103	0.0766	630.77	789.42	833.65	884.62
2	0.3447	0.0102	2.96		0.0607	0.1007	0.1037	0.1071	0.1072	0.1074	0.0875	495.10	887.25	916.67	950.00	950.98	952.94	757.84		
3	0.6036	0.0226	3.74	3-5	0.0704	0.1087	0.1344	0.1438	0.1472	0.1476	0.1339	211.50	380.97	494.69	536.28	551.33	553.10	492.48		
4	0.5606	0.0243	4.33		0.0886	0.1136	0.1483	0.1824	0.1825	0.1826	0.1667	264.61	367.49	510.29	650.62	651.03	651.44	586.01		
5	0.6594	0.0359	5.44	5-10	0.0997	0.1002	0.171	0.1757	0.1803	0.1808	0.1776	177.72	179.11	376.32	389.42	402.23	403.62	394.71		
6	0.5792	0.0448	7.73		0.0942	0.1152	0.1972	0.1972	0.197	0.1917	0.1802	110.27	157.14	340.18	340.18	339.73	327.90	302.23		
7	0.5482	0.0554	10.11	10-15	0.0987	0.1112	0.1556	0.1647	0.1653	0.1632	0.1621	78.16	100.72	180.87	197.29	198.38	194.58	192.60		
8	0.5079	0.0697	13.72		0.1008	0.1075	0.167	0.1885	0.199	0.2031	0.2002	44.62	54.23	139.60	170.44	185.51	191.39	187.23		
9	0.3091	0.0574	18.70	15-20	0.1398	0.1447	0.1493	0.1558	0.1876	0.2056	0.1989	143.55	152.09	160.10	171.43	226.83	258.19	246.52		
10	0.4672	0.0788	16.87		0.1443	0.1765	0.1887	0.225	0.2412	0.255	0.2412	120.69	123.98	139.47	185.53	206.09	223.60	206.09		
11	0.836	0.1757	21.02	20-25	0.2215	0.3162	0.3427	0.4153	0.4009	0.3672	0.3671	26.07	79.97	95.05	136.37	128.17	108.99	108.94		
12	0.7081	0.1658	25.41		0.2975	0.3901	0.4374	0.442	0.4376	0.4246	0.4234	79.43	135.28	163.81	166.59	163.93	156.09	155.37		
13	1.083	0.3024	27.92	25-30	0.4789	0.5719	0.5849	0.5865	0.6078	0.6468	0.6466	58.37	89.12	93.42	93.95	100.99	113.89	113.82		
14	0.8873	0.2617	29.49		0.413	0.5463	0.596	0.5963	0.5987	0.6054	0.6053	57.81	108.75	127.74	127.86	128.77	131.33	131.30		

### **4. %Average DI water reabsorption (Dry basis) of different moisture content BC samples**

Moisture	% Reabsorption at different time intervals							SD of% Reabsorption at different time intervals						
	2h	4h	6h	20h	24h	28h	48h	2h	4h	6h	20h	24h	28h	48h
2-3	562.93	838.34	875.16	917.31	919.72	921.66	697.19	95.93	69.18	58.70	46.23	44.21	44.23	85.78
3-5	238.06	374.23	502.49	593.45	601.18	602.27	539.24	37.55	9.53	11.03	80.85	70.50	69.54	66.14
5-10	143.99	168.13	358.25	364.80	370.98	365.76	348.47	15.53	25.56	34.82	44.19	53.54	53.54	65.39
10-15	61.39	77.48	160.23	183.87	191.94	192.99	189.92	23.72	32.87	29.18	18.98	9.10	2.26	3.80
15-20	132.12	138.04	149.79	178.48	216.46	240.90	226.30	16.17	19.87	14.59	9.97	14.66	24.45	28.58
20-25	52.75	107.62	129.43	151.48	146.05	132.54	132.15	37.74	39.12	48.62	21.37	25.29	33.30	32.83
25-30	58.09	98.94	110.58	110.90	114.88	122.61	122.56	0.39	13.88	24.27	23.98	19.64	12.34	12.36

## APPENDIX H

### Mechanical Properties and % Re-absorption Capacities of BC Samples at Swollen State with Time

#### 1. 25-30% moisture content (wet BC) BC samples in DI water for 48 hrs.

Time/h	Trial 1		Y /MPa	Trial 2		Y /Mpa	Avg, Reabsorption		Avg Y /MPa	Standard deviation		
	% Reabsorption			% Reabsorption			Dry basis	Wet basis		Reab.dry basis	Reab.wet basis	Y/MPa
	Dry Basis	Wet basis	Dry Basis	Wet basis								
0			30.67			53.48	none	none	42.08			16.13
4	97.06	49.25	23.39	92.12	45.95	50.98	94.59	48.60	37.19	3.49	0.92	19.51
20	138.96	58.15	22.64	154.44	60.70	37.14	146.70	59.43	29.89	10.94	1.80	10.25
24	174.76	63.60	18.57	172.65	63.32	33.17	173.70	63.46	25.87	1.49	0.20	10.32
48	187.30	65.19	17.19	188.86	65.38	26.95	188.08	65.29	22.07	1.11	0.13	6.90

#### 2. 25-30% moisture content(wet BC) BC samples in saline for 48 hrs

Time/h	Trial 1		Y /Mpa	Trial 2		Y /Mpa	Avg, Reabsorption		Avg Y /MPa	Standard deviation		
	% Reabsorption			% Reabsorption			Dry basis	Wet basis		Reab.dry basis	Reab.wet basis	Y/MPa
	Dry Basis	Wet basis	Dry Basis	Wet basis								
0			66.73			67.7	none	none	67.22			0.69
4	89.82	47.32	59.41	76.80	43.44	58.27	83.31	45.38	58.84	9.21	2.74	0.81
20	92.34	48.01	44.14	110.10	52.40	51.5	101.22	50.21	47.82	12.56	3.11	5.20
24	111.43	52.70	40.69	106.15	51.49	36.32	108.79	52.10	38.51	3.73	0.86	3.09
48	130.00	56.52	39.84	119.59	54.46	25.42	124.79	55.49	32.63	7.36	1.46	10.20

**3. 2-3% moisture content (dry BC) BC samples in DI water for 48 hrs.**

Time /h	Trial 1			Trial 2			Avg, Reabsorption		Avg Y /MPa	Standard deviation		
	% Reabsorption		Y /MPa	% Reabsorption		Y /MPa	Dry basis	Wet basis		Reab.dry basis	Reab.wet basis	Y/MPa
	Dry Basis	Wet basis		Dry Basis	Wet basis							
0			548.53			660.24	none	none	604.39			78.99
4	795.21	88.83	160.06	990.50	90.83	152.77	892.85	89.83	156.42	138.09	1.41	5.15
20	938.08	89.65	97.26	1215.90	92.40	83.50	1076.99	91.02	90.38	196.45	1.95	9.73
24	1281.06	92.76	88.55	1366.29	93.18	79.36	1323.67	92.97	83.96	60.26	0.30	6.50
48	1357.52	93.14	77.78	1396.58	93.32	45.09	1377.05	93.23	61.44	27.62	0.13	23.12



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**4. 2-3% moisture content (dry BC) BC samples in saline for 48 hrs.**

Time/h	Trial 1			Trial 2			Avg, Reabsorption		Avg Y /MPa	Standard deviation		
	% Reabsorption		Y /MPa	% Reabsorption		Y /MPa	Dry basis	Wet basis		Reab.dry basis	Reab.wet basis	Y/MPa
	Dry Basis	Wet basis		Dry Basis	Wet basis							
0			691.97			621.42	none	none	656.70			49.89
4	824.44	89.18	339.87	863.18	89.62	104.07	843.81	89.40	221.97	27.39	0.31	166.74
20	906.00	90.06	246.91	880.95	89.81	83.54	893.48	89.93	165.23	17.71	0.18	115.52
24	891.67	89.92	160.25	1018.48	91.06	66.09	955.07	90.49	113.17	89.67	0.81	66.58
48	1166.67	92.11	152.41	1023.77	91.10	58.42	1095.22	91.60	105.42	101.04	0.71	66.46