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AGRIMA

Bacterial cellulose production by the bacterium *Komagateibacter rhaeticus* using different nitrogen sources, and different C/N ratios

Summer Camp | July 7 – 11, 2025 | Marseille, France

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Agri-food Waste Management for Sustainable bio-economy through Higher Education curricula and upskilling

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Goal

AGRIMA aims to foster universities' **capacity building** for the **green transition** through **innovative practices and higher education curricula updating in agri-food waste management** to the **circular bioeconomy**.

AGRIMA addresses:

1. **Advancing pedagogical methods** for industrial agri-food waste valorisation **based on business-academia synergies**.
2. **Integrating citizen science** in bio-economy-enhanced waste valorisation as a means of **civic engagement and environmental advocacy**.

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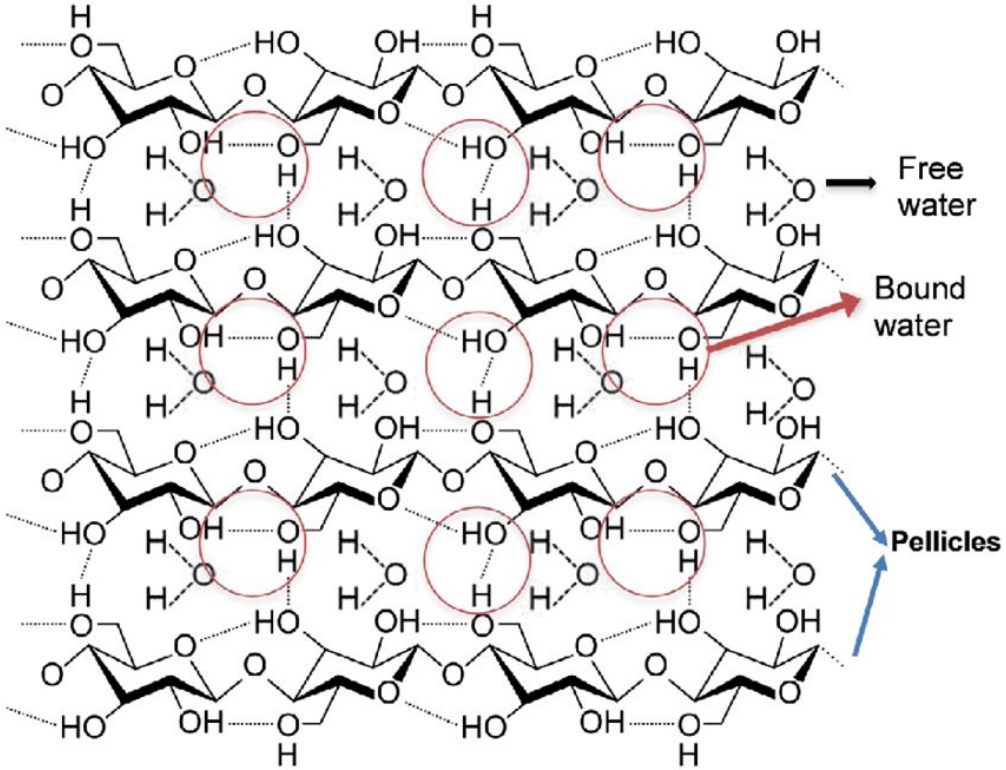
Bacterial Cellulose

- It’s a natural polymer made by certain bacteria.

- The chemical structure of BC is the same as the cellulose from plants or algae → Composed of glucose units linked by β-1,4-glycosidic bonds



Which Bacteria produce Cellulose?

Bacterial genera of:

- *Acetobacter,*
- *Gluconobacter,*
- ***Komagataeibacter,***
- *Agrobacterium,*
- *Sarcina,*
- *Azotobacter,*
- *Rhizobium*
- *Alcaligenes*

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Bacterial Cellulose Properties

- High purity ⟶ ✖ lignin or hemicellulose
- High water holding capacity
- High degree of polymerization
- High mechanical strength
- High crystallinity

Some uses of Bacterial Cellulose:

- Food packaging
- Water bioremediation (adsorption of heavy metals and flocculation capacity)
- Biomedicine (tissue engineering and controlled drug delivery)
- Additive manufacturing (3D Printing)
- Electronics and biosensors (enhanced conductivity)

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Sources for the production of the BC

1. **Synthetic Sources**

Carbon Sources:

- **Glucose**
- Fructose
- Sucrose
- Glycerol

Nitrogen Sources:

- **Peptone**
- **Yeast Extract**
- **Ammonium sulfate (NH4)2SO4(NH4)2SO4**
- Ammonium chloride (NH4Cl)(NH4Cl)
- **Urea**
- Sodium nitrate ($NaNO_3$)

2. **Other sources (wastes from Food Industry)**

- Fruit and Vegetable Processing Wastes
- Molasses
- Whey (from cheese production)
- Beer brewery waste

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Purpose of the study

- The aim of this study is to observe the growth of Bacterial Cellulose with the bacterium *Komagateibacter rhaeticus* UNIWA AAK2, using in culture different nitrogen sources, namely Peptone, Urea and Ammonia Sulphate.

- Then the fermentations were carried out again with an increased C/N=30

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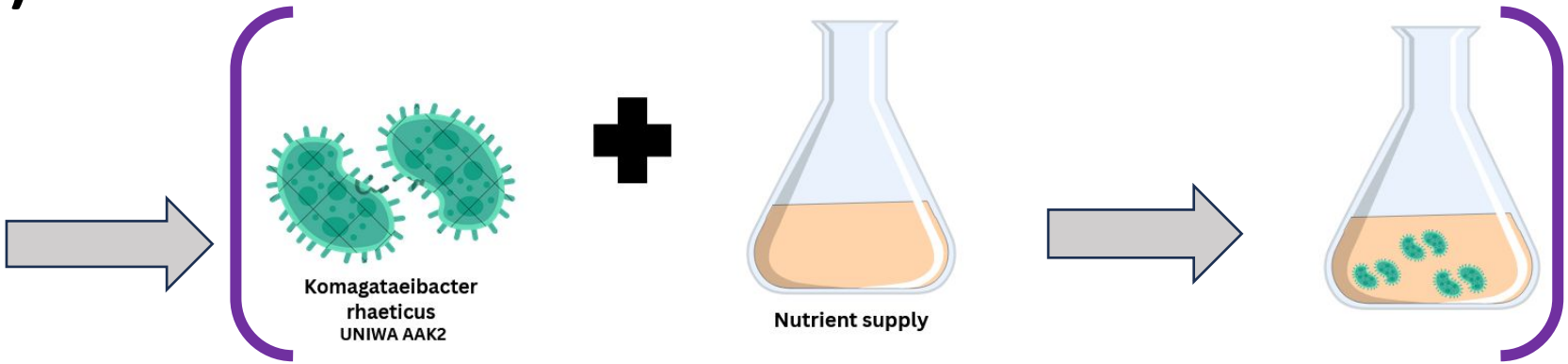
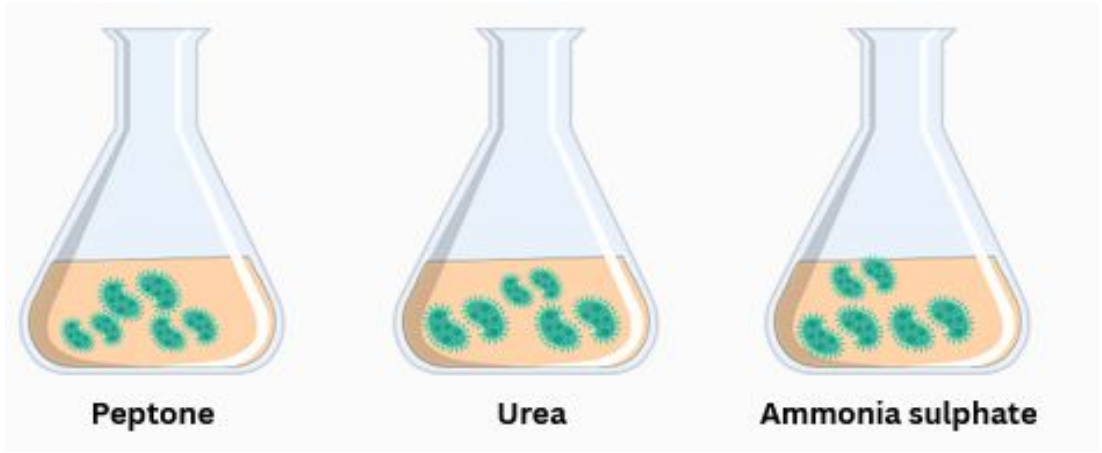
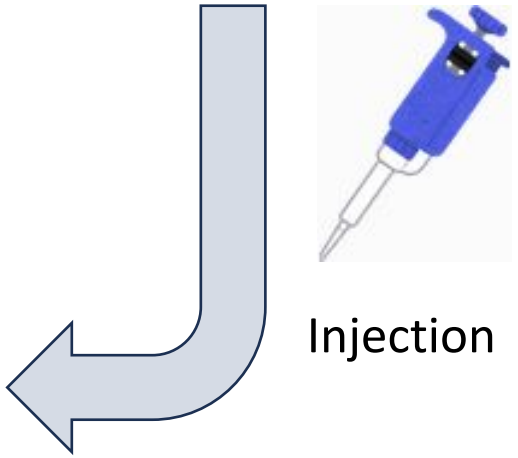
Methodology (1)

Pre-Cultivation



- 48 Hours non-static fermentation
- T= 30oC
- pH= 5,5 - 6





Injection

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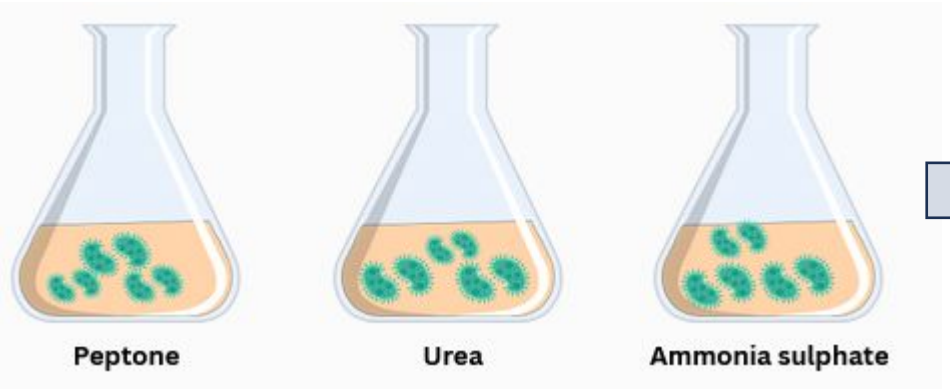
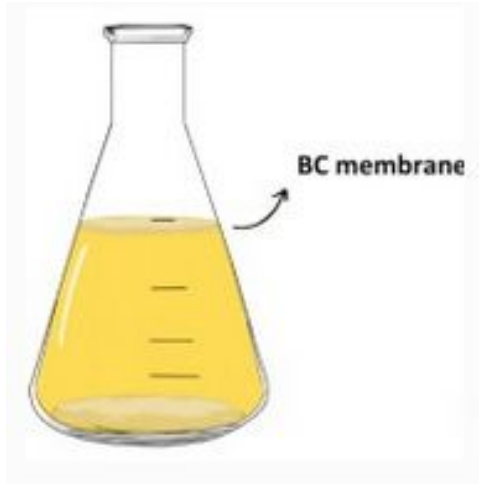
Methodology (2)



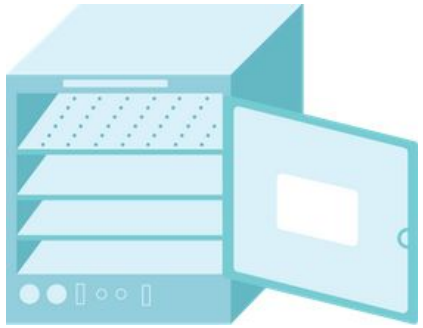
Static Fermentation
pH = 5.5 - 6.0
T = 30oC



- Harvesting BC membrane
- Immersion in 1M NaOH at 80^{o}C for 115 min.

Methodology (3)

After cleaning the BC



OvenDrying

spectrophotometer

Determination of carbon source consumption (DNS method)

Determination of nitrogen source consumption (FAN method)

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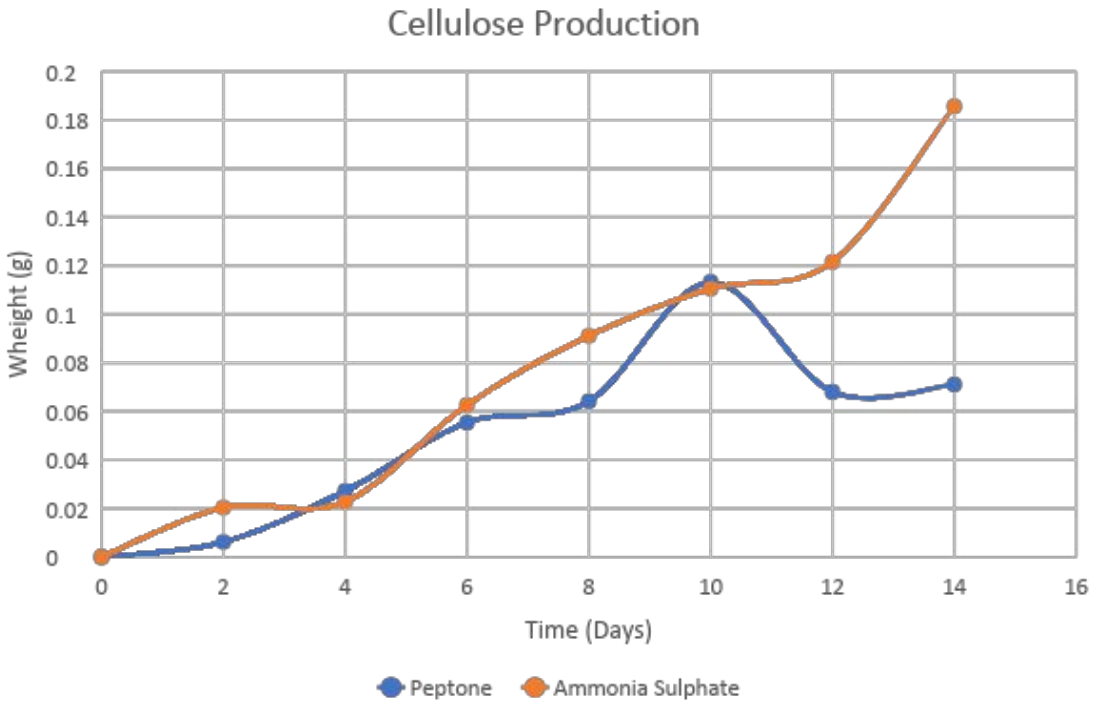
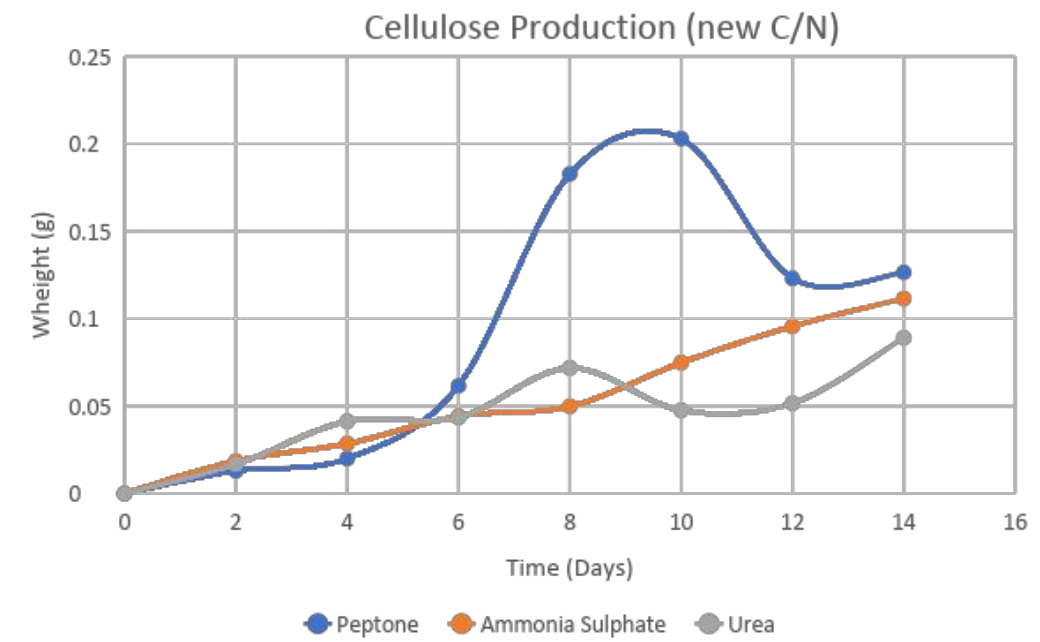
Results (1)

Peptone		Ammonia Sulphate		Urea	
Time (Days)	Cellulose (g)	Time (Days)	Cellulose (g)	Time (Days)	Cellulose (g)
0	0	0	0	0	0
2	0,0062	2	0,0204	2	0
4	0,0272	4	0,0227	4	0
6	0,0554	6	0,0626	6	0
8	0,0641	8	0,0911	8	0
10	0,1133	10	0,1105	10	0
12	0,068	12	0,1215	12	0
14	0,0711	14	0,1856	14	0



Peptone C/N ≈ 30		Ammonia Sulphate C/N ≈ 30		Urea C/N ≈ 30	
Time (Days)	Cellulose (g)	Time (Days)	Cellulose (g)	Time (Days)	Cellulose (g)
0	0	0	0	0	0
2	0,013	2	0,0186	2	0,0167
4	0,02	4	0,0286	4	0,0413
6	0,0616	6	0,0442	6	0,0435
8	0,1828	8	0,0498	8	0,0718
10	0,203	10	0,0748	10	0,0476
12	0,123	12	0,0956	12	0,0516
14	0,1265	14	0,1115	14	0,0891



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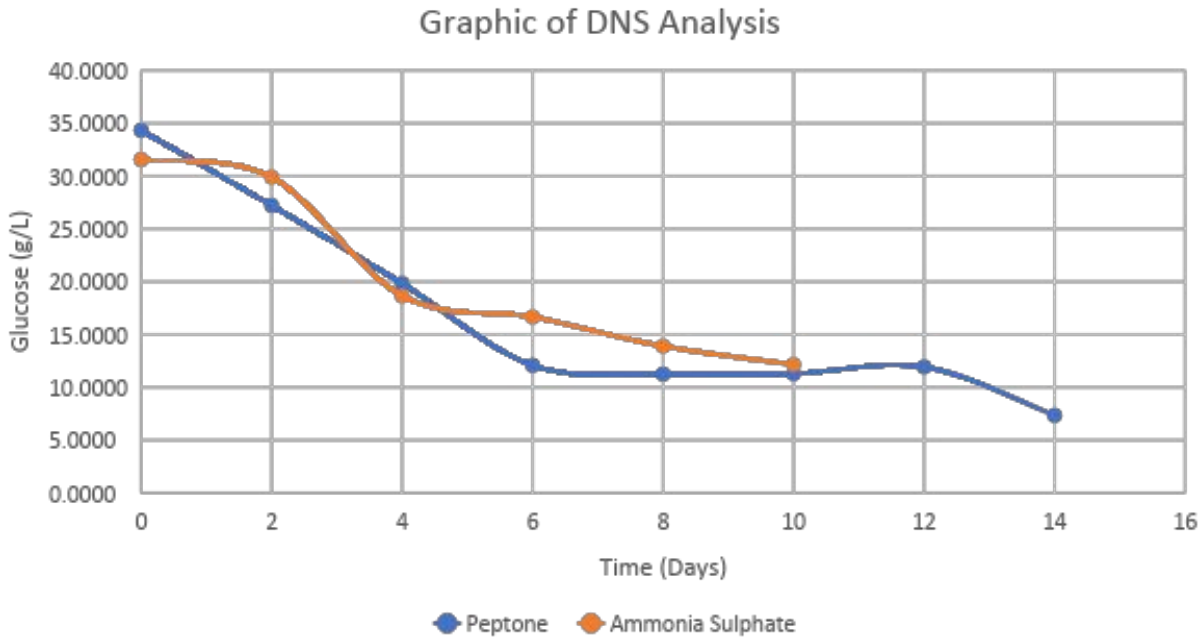
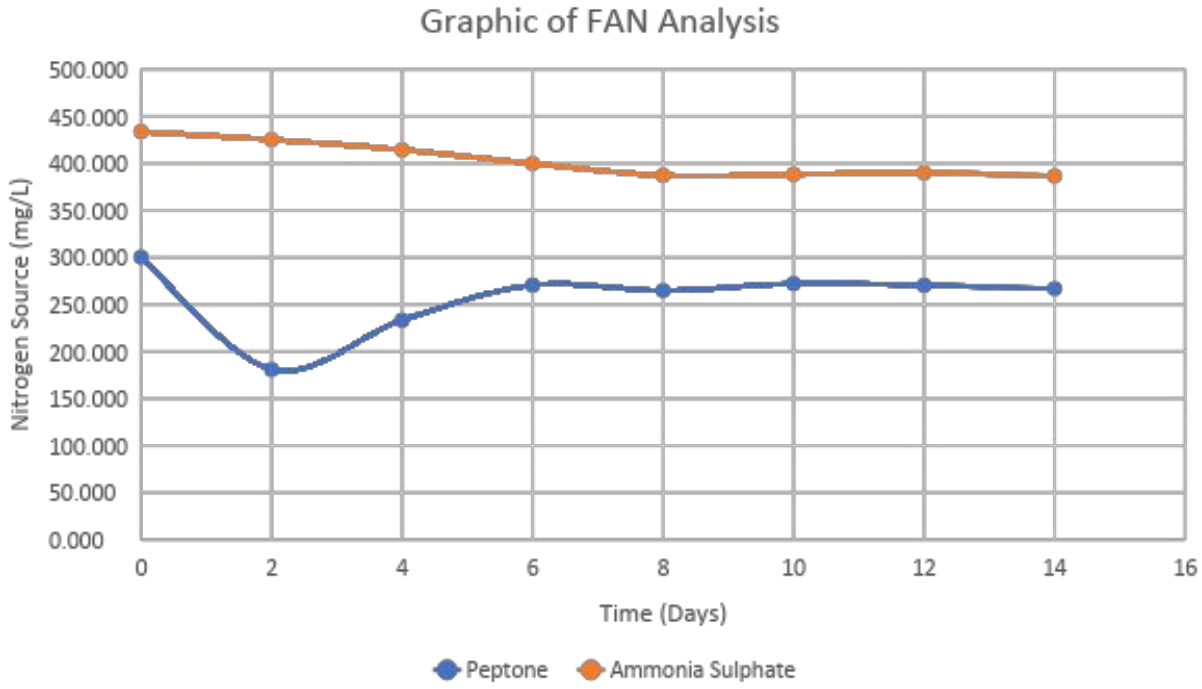
Results (2)

	Time (Days)	N sources (mg/L)	Glc (g/L)	Cellulose (g)
Peptone	0	300,389	34,350	0
	2	180,972	27,218	0,0062
	4	233,396	19,838	0,0272
	6	270,688	12,087	0,0554
	8	264,884	11,261	0,0641
	10	272,239	11,300	0,1133
	12	270,453	11,938	0,068
	14	266,928	7,352	0,0711

	Time (Days)	N sources (mg/L)	Glc (g/L)	Cellulose (g)
Ammonia Sulphate	0	433,789	31,5601	0
	2	425,228	29,8999	0,0204
	4	414,534	18,6494	0,0227
	6	399,898	16,6580	0,0626
	8	387,33	13,9018	0,0911
	10	388,455	12,1586	0,1105
	12	389,98	21,8838	0,1215
	14	386,656	25,6955	0,1856

	Time (Days)	N sources (mg/L)	Glc (g/L)	Cellulose (g)
Urea	0	193,8962	33,2126	0
	4	204,1884	30,6098	0
	6	187,6928	25,3674	0
	8	79,1366	2,9667	0
	10	166,1967	8,1209	0
	12	177,1938	8,3297	0
	14	174,2918	8,3342	0





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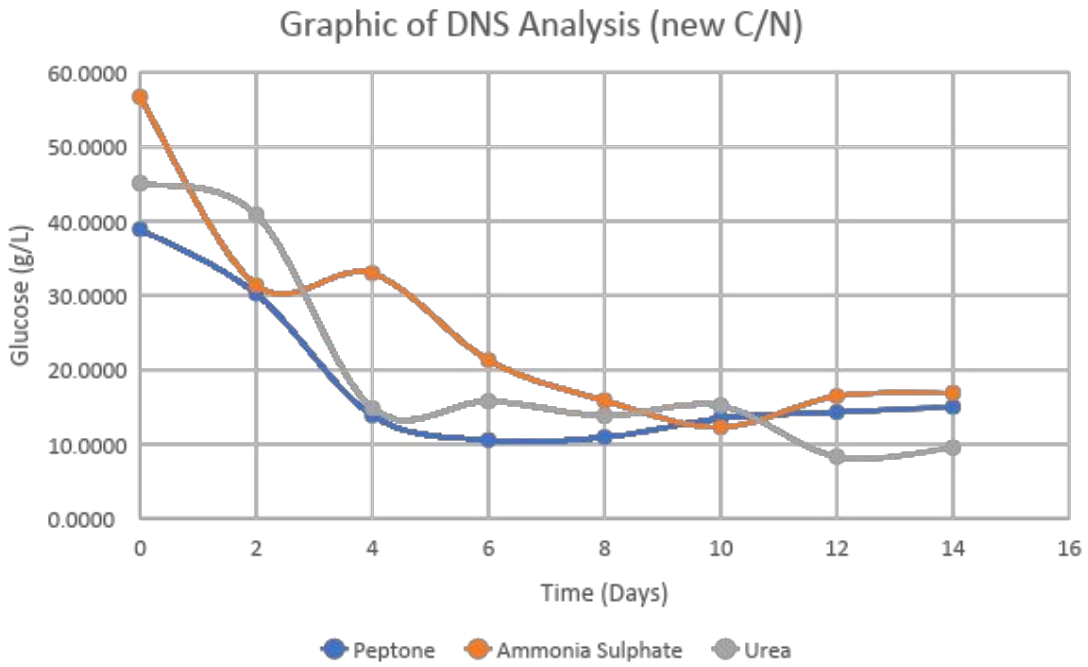
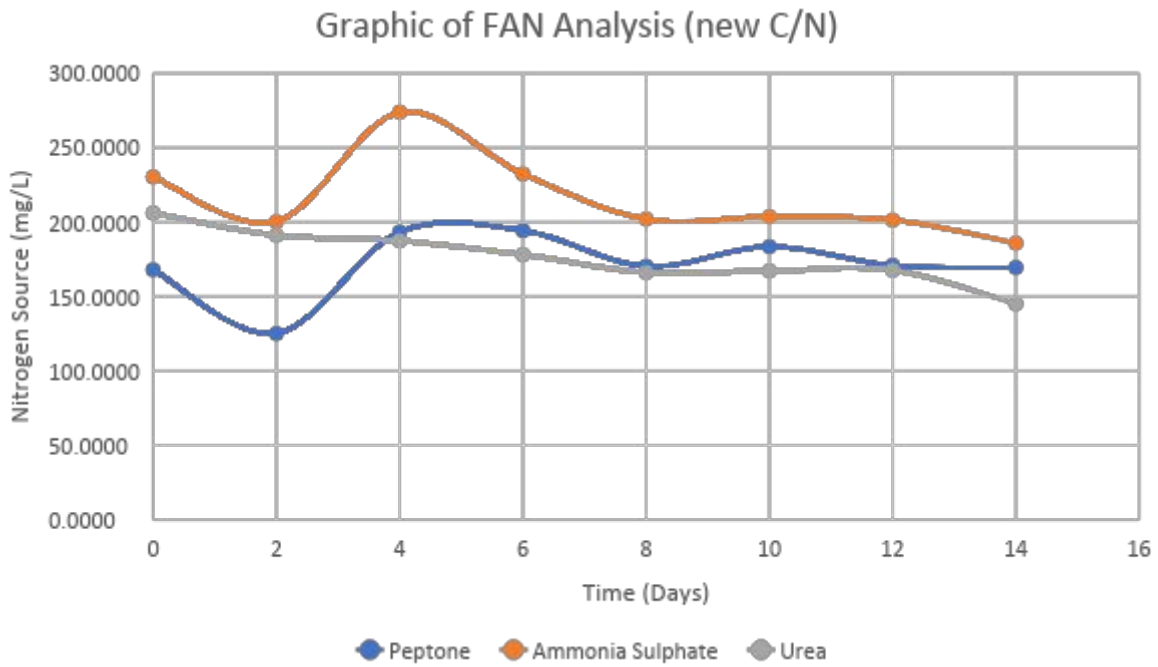
Results (3)

	Time (Days)	N sources (mg/L)	Glc (g/L)	Cellulose (g)
Peptone C/N ≈ 30	0	167,9779	38,9275	0
	2	125,0706	30,2439	0,013
	4	193,3793	13,8765	0,02
	6	194,3192	10,5563	0,0616
	8	170,3089	10,9328	0,1828
	10	183,5265	13,4735	0,203
	12	170,9081	14,3170	0,123
	14	169,4629	15,0145	0,1265

	Time (Days)	N sources (mg/L)	Glc (g/L)	Cellulose (g)
A. Sulphate C/N ≈ 30	0	230,3181	56,6817	0
	2	200,2877	31,3519	0,0186
	4	273,9069	32,9924	0,0286
	6	232,4095	21,3101	0,0442
	8	202,0429	15,8668	0,0498
	10	203,6290	12,2884	0,0748
	12	201,3967	16,4631	0,0956
	14	185,8645	16,8925	0,1115

	Time (Days)	N sources (mg/L)	Glc (g/L)	Cellulose (g)
Urea C/N ≈ 30	0	206,0682	45,1080	0
	2	191,1705	40,8862	0,0167
	4	187,4578	14,8366	0,0413
	6	178,1220	15,7861	0,0435
	8	166,1850	13,8480	0,0718
	10	167,1954	15,1776	0,0476
	12	167,7006	8,3252	0,0516
	14	145,1144	9,5345	0,0891





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Conclusions

- We observe that in the first fermentations the use of Ammonium sulphate had better bacterial cellulose production than the use of peptone.
- Also in the first fermentation the microorganism failed to produce cellulose with urea as the nitrogen source, although the microorganism had grown normally
- Another observation is that in the new fermentations (C/N = 30) the production of cellulose by the micro-organism was not so significantly high
- In all fermentations not all nutrients were fully consumed, especially in the nitrogen source its consumption was very low
- According to the results both peptone and ammonia sulphate are very good sources of nitrogen for this microorganism, although in the first fermentations ammonia sulphate is the dominant one

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This study was conducted at the University of the Aegean, within the Laboratory of Physicochemical & Biotechnological Utilization of Food By-products in Lemnos. The authors gratefully acknowledge the guidance of Dr. Dimitris Sarris, contributions from Dr. Danai Ioanna Koukoumaki.

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Thank you !

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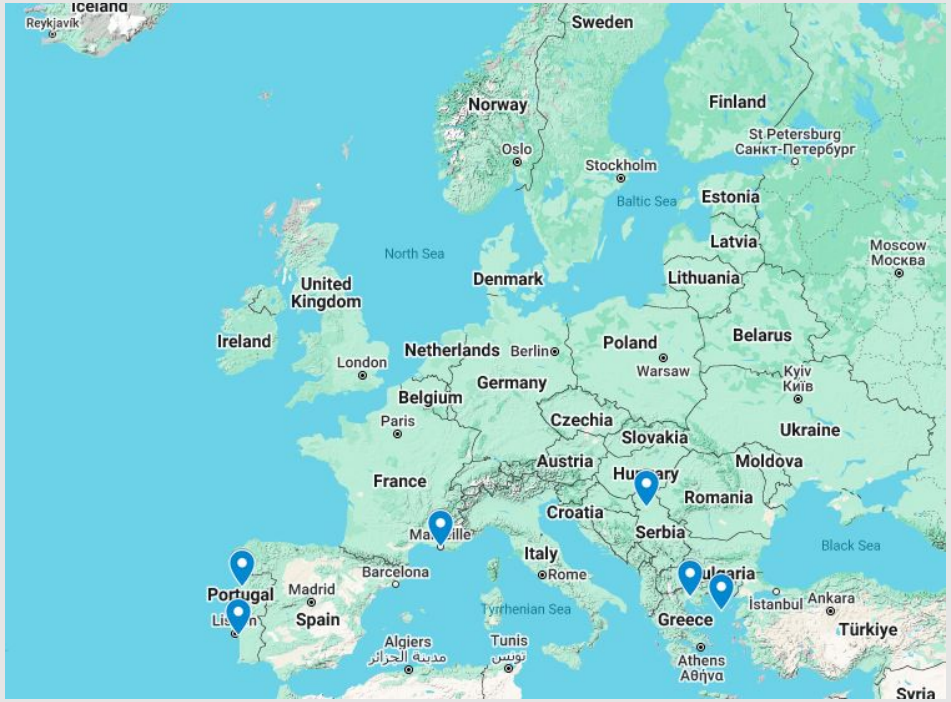

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