

How to Cite:

Ahmed, E. F., Ali, W. S., & Heider, N. H. (2022). Determination the optimal conditions for production of nanocellulose from acetic acid bacteria. *International Journal of Health Sciences*, 6(S4), 9543–9557. <https://doi.org/10.53730/ijhs.v6nS4.10817>

Determination the optimal conditions for production of nanocellulose from acetic acid bacteria

Entissar Faroun Ahmed

Department of Biology/College of Science/University of Baghdad/Baghdad/Iraq
Corresponding author email: entissar.ahmed1102@sc.uobaghdad.edu.iq

Wala'a Shawkat Ali

Department of Biology/College of Science/University of Baghdad/Baghdad/Iraq

Nadhem Hasan Heider

Department of Biotechnology/College of Science/University of Baghdad/Baghdad/Iraq

Abstract---Bacteria could produce bacterial nanocellulose through a procedure steps: polymerization and crystallization, that occur in the cytoplasm of the bacteria, the residues of glucose polymerize to (β -1,4) lineal glucan chains that produced from bacterial cell extracellularly, these lineal glucan are converted to microfibrils, after that these microfibrils collected together to shape very pure three dimensional pored net. It could be obtained a pure cellulose that created by some M.O, from the one of the active producer organism like Acetic acid bacteria (AAB), that it is a gram -ve, motile and live in aerobic condition. The bacterial nanocellulose (BNC) have great consideration in many fields because of its flexible properties, features and advantages of the BNC. So in this study, the acetic acid bacteria (5AC) isolate was isolated from apple vinegar and detected the ability of this isolate to produce the nanocellulose on the Hestrin-Schramm (HS) medium, and then optimized the production condition, four parameters were investigated: temperature, pH, different carbon sources and nitrogen sources. After incubation the isolate for about (168- 192) hrs, the highest yield of BNC was identified by determining the wet weight, dry weight also the consumed sugar for each parameter. The results showed that the optimum temperature was (30°C), the optimum pH was (5), the optimum carbon source was the glucose while peptone and yeast extract together showed the best and optimum nitrogen sources for production of BNC, highest results for the produced BNC from the isolate acetic acid bacteria (5AC) were: wet weight (0.96gm), dry weight (0.055gm) and the consumed sugar (80%).

Keywords---bacterial nanocellulose, acetic acid bacteria, optimum conditions, HS broth, glucose.

Introduction

The cellulose is an abundant complex on earth which estimated by 50% of the produced mass in the plants by the process of photosynthesis, recurrence lineal chain of (β -1,4) that connected the units of D-glucose in cellulose is caused a homo-polymer complex also the molecular formula of cellulose known as $(C_6H_{10}O_5)_n$, the cellulose could be utilized as bio polymer as a try for substitute petro-chemicals derivative polymer because of anxiety to the environment nature and in addition to that cellulose has extensive use in many different fields for example in: food, medicinal fields and textiles fields and the greatest reported use of cellulose was in the paper also in pulp manufacturing, nowadays the turnout for production of bacterial cellulose (BC) is an alternative way for plant cellulose the (BC) production is a procedure that may be influenced by many variables and need optimal conditions to reach maximum production (Sathar, 2012). BC has a distinctive form of nanofibers with a diameter of nearly (50-100) nm, it is secreted by particular bacteria extracellularly, at most by the strains of *Gluconacetobacter* (Zuppolini *et al.*, 2022). Its exceptional fibrillar nano-structure gives it with great physical and mechanical properties like: high porosity, very fine reticulated structure, highly tensile strength, also great crystallinity and hydrophilicity, even though its chemical structure is analogous to plant cellulose (Goldberg *et al.*, 2015). BC nano-fibers with a (3D) net organization (pellicle) are hopeful resources for different applications, nano-fibrillated BC has low dis-persibility in organic solvents and diverse approaches such as hydrophobic surface treatments are used to alter their properties (Ross *et al.*, 1991). The important feature of AAB is their capability to produce extracellular polymers like bacterial cellulose which is mostly synthesized by the genera species of the *Komagataeibacter* also *Gluconacetobacter*, the BC biopolymer is multi use with greatly exclusive properties for example: highly (H_2O)-holding capability, biocompatibility, ultrafine networks organization, highly crystallinity which gives and assistance a variety of commercial usage like wounds dressing, in tablet preparation also in functional food additive (Cacicedo *et al.*, 2016; Gomes *et al.*, 2018). Bacterial cellulose conserve the cells organism from the destruction by UV light and helps in moisture retention to avoid drying of the natural materials on which the bacteria are grown on, the passageway to yield cellulose from D-glucose by *K. xylinus* contains of (4) enzymatic steps, the enzymes complicated in cellulose biosynthesis are: glucose kinase, phosphor-glucomutase, Uridine di-phosphate glucose-pyrophosphorylase (UDPG) and membrane bound cellulose synthase (Donini *et al.*, 2010). The serious features affecting the bacterial cellulose construction comprise the composition of fermentation elements such as: C sources, N sources and minerals sources in the culture medium also the physical circumstances like: pH, Temperatures, O_2 dissolved in the medium, the ratio of inoculation and age of the inoculum (Aswini *et al.*, 2020). This study aimed to determine some optimal conditions including; temperature, pH, C and N sources for producing nanocellulose by acetic acid bacteria.

Materials and Methods

Culture media

The GY broth medium was prepared according to (Trinh *et al.*, 2016), it was used for isolation, activation and maintenance of the bacterial isolates. While, Hestrin-Schramm (HS) medium was prepared according to (Abol-Fotouh *et al.*, 2020) and it was used for detection of production of nanocellulose from the bacterial isolates.

Samples collection and isolation of (AAB)

Total 25 samples of apple vinegar and date palm vinegar were collected under aseptic condition from local markets in Baghdad city. Acetic acid bacteria (AAB) were isolated and identified according to procedures of (Bellankimath *et al.*, 2017) and the identification of bacterial isolates was confirmed by morphological and biochemical tests.

Screening the ability of Acetic Acid Bacteria (AAB) for production of bacterial nanocellulose (BNC):

AAB isolates were activated in GY broth medium for 18-24hrs. at 30°C, after that, 1.5% from this activated broth was inoculated into (100ml screwed cups) contains 50ml of HS broth, cups statically incubated at 30°C for 7-8 days. After incubation period the bacterial isolates that able to produce cellulose in broth were determined (Costa *et al.*, 2017; Abol-Fotouh *et al.*, 2020) and the best isolate of production was selected and used in the subsequent experiments.

Measurement procedures for produced BNC

Wet weight method

After formation or production of BNC in the medium, the membrane layer was taken and washed by D.W and then the wet weight /gm was measured by electronic balance.

Dry weight method

After the measuring of the wet weight of the BNC, membrane was left to dry for about (2-3) days at room temperature (R.T) after that the dry weight/gm was determined by electronic balance.

Estimation of consumed sugar

Sugar of the media measured by refractometer, the method was performed by measuring about 0.3ml from control (media without bacteria) and recording the origin sugar value. For the inoculated media the method was performed by taken about 1ml of the medium after incubation period (bacterial growth) then centrifuged the sample for 5min. at 5000rpm, after that measuring about 0.3ml from the sample (free of the cells) and recording the remain sugar value after

bacterial growth, the differences between the two values giving an indication on the value of consumed sugar (%) (Abol-Fotouh *et al.*, 2020; Abouelkheir *et al.*, 2020).

Optimization and effects of various culture conditions on production of BNC

The following experiments were performed with duplicates. The control in each experiment was represented the medium without inoculum:

Effect of temperature:

Fifty ml from sterilized Hestrin-Schramm (HS) broth was inoculated by 1ml overnight activated bacterial culture broth and incubated at different temperatures (25°C, 30°C, 35°C, 37°C and 40°C) for 8 days, after incubation period: the wet weight, dry weight and consumed sugar were determined.

Effect of pH

Fifty ml from sterilized Hestrin-Schramm (HS) broth was inoculated by 1ml overnight activated bacterial culture broth then incubated at diverse pH values (4, 5, 6, 7 and 8) and incubated at 30°C for 8days, after incubation period: the wet weight, dry weight and consumed sugar were estimated.

Effect of different carbon (C) source

Different C sources include: (Fructose, Sucrose, Glucose, Maltose, Starch, Cellulose and Glycerol) sterilized by millipore filter unit (0.2µm) and then added to 50 ml of sterilized HS broth medium with pH 5, then inoculated with 1ml of overnight activated bacterial culture broth and incubated at 30°C for 8 days, after incubation period: the wet weight, dry weight and consumed sugar were estimated.

Effect of different nitrogen source

Fifty ml of sterilized HS broth medium (pH 5) containing: glucose (as a carbon source) and different nitrogen sources include: [NH₄Cl, (NH₄)₂SO₄, Yeast extract, Peptone, (Peptone + Yeast extract) and Urea] was inoculated with 1 ml of overnight activated bacterial culture broth and incubated at 30°C for 8days, after this period: the wet weight, dry weight and consumed sugar were determined.

Results and Discussion

Samples collection and isolation of acetic acid bacteria (AAB)

Twenty five samples were collected from different vinegar types (apple and palm date vinegar), five isolates were characterized as acetic acid bacteria (AAB) from the total samples. The bacterial colony observed as: aerobic, round, smooth, raised with white or off white color. Microscopic examination of the isolates displayed that they were all: Gram -ve, non-produced spore and rod. Results of

biochemical tests showed that all isolates are negative oxidase test and positive catalase test, motile and produce cellulose.

The acetic acid bacteria are gram -ve, bacilli-shaped and can appear as single cell, in couples or in chains, motile because the presence of flagella which can be either polar or peritrichous, do not form spores, they have an strict aerobic metabolism, that the O₂ as terminal electron acceptor (Holt *et al.*, 1994). Bacterial isolates were recognized by observation of the morphology of its colony, Gram reaction, series and motility tests. The morphology of its cell was examined by using SEM, for identification by biochemical procedure, an estimation of different experiments involving: catalase, oxidase production and fermentation various carbohydrates (Thongwai *et al.*, 2022).

BNC produced by acetic acid bacteria

The BC produced by acetic acid bacteria known as generally recognized as safe (GRAS) food by US Food and Drug Administration (FDA) (Thongwai *et al.*, 2022). In this study, the bacterial isolates that have the ability to produce bacterial nanocellulose (BNC) were detected after the incubation at pH (6.5-7), 30°C, inoculum size 2% and incubation period (7-8) days in HS medium, the positive results was observed as a whit thick membrane layer at the surface of the liquid medium with diameter of about 48 mm approximately (Figure 1), the bacterial isolate (acetic acid bacteria 5AC) was selected as best BNC producer after screening.

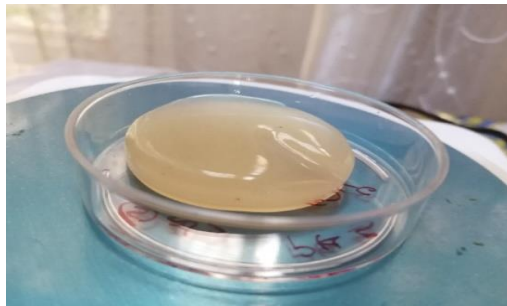


Figure (1) Bacterial nanocellulose membrane (wet membrane) produced by acetic acid bacteria in Hestrin-Schramm (HS) medium

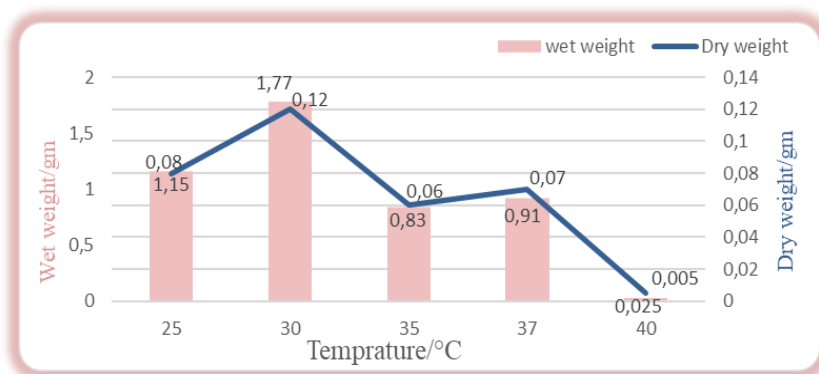
Hestrin-Schramm broth is favorable medium to get high productivity of BC from AAB. Thongwai *et al.* (2022) reported that HS broth pH (6.0), inoculated by 5% (v/v) from the strain *Komagataeibacter maltaceti* P285 then incubated at temperature 30°C for about 7 day, after cultivation, cellulose harvested then purified with high productivity. This medium was also used by Zielińska *et al.* (2022) to produce high amounts of BC from another strain of AAB; *K. xylinus*. The nanocellulose termed as bacterial cellulose (BC) which produced through the bottom up approach use the fermentation of low mwt. sugar by cellulose creating bacteria, such as *Acetobacter* spp. (Ross *et al.*, 1991; Iguchi *et al.*, 2000), it's a pure cellulose without existence of pectin or lignin, this cellulose is created by the bacteria into the liquid culture medium as nano-fibers with a diameter from about (25-100) nm, then these nano-fibers makes up the bacterial cellulose

pellicles membrane in the liquid medium (Lee *et al.*, 2012). When glucose sugar used as a C source such as in HS medium for example or any other medium with similar C source, the bacterial nano-cellulose production occurs in four enzymatic stages: (I) The phosphorylation of glucose to (Glc-6-P) through glucokinase, (II) Isomerization of Glc-6-P to Glc-1-P by phosphoglucomutase , (III) Creation of uridine diphosphate glucose (UDP- glucose) or (UDPGlc) that is a direct precursor of cellulose through UDPG pyro-phosphorylase , (IV) Polymerization of (UDP glucose) to (β -1, 4) glucan chains by the cellulose synthase enzyme complex (Ross *et al.*, 1987; Barja, 2021).

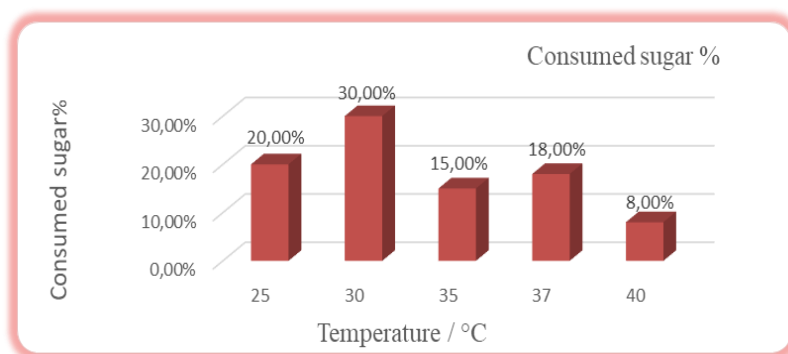
Optimization and the Effects of various culture conditions on production of (BNC)

Effect of temperature

From incubation of bacteria in different temperatures (25°C, 30°C, 35°C, 37°C and 40°C) for 8days, the highest production of BNC from acetic acid bacteria (5AC) isolate was observed at 30°C depending on the results of: wet weight (1.77gm), dry weight (0.12gm) and consumed sugar (30%) (Figure 2, A and B). It was important to know that AAB produced bacterial nanocellulose in a wide range of temperatures (25-40) °C, production of BNC in relatively high temperature (37°C) in spite of the low production but may indicate that this type of bacteria can grow at this temperature and the BNC could be produced in this levels of temperature, this property may be favored in industrial application since it reduce the cost of cooling.



-A-



- B -

Figure (2) Effect of temperatures on bacterial nanocellulose production from acetic acid bacteria isolate (5AC) in HS medium, A- Wet and dry weight/gm., B- Consumed sugar (%)

Sijabat *et al.* (2020) investigated that the environmental conditions represent a key role in the microorganism physiology and BNC production from AAB spp. was found to be affected by this environmental conditions, when the samples incubated for 10 days at (R.T), it was showed that the bacterial nano-cellulose surface is smooth in spite of some bumpy also irregular parts, there was a obvious growth of fungi or other impurities which can affect the growing of bacteria that yield bacterial nano-cellulose. Some researchers showed that the temperature could play an important part in bacterial cellulose production, also it was observed that the culture of *A. xylinum* require a static-warm circumstance with temperature not below (20°C) also not higher than (30°C) the best temperatures among (23-30)°C (Teoh *et al.*, 2004). The production of cellulose by *A. senegalensis* explain that the interaction of temperature with other condition such as pH improved the production of BC to the maximum at temperature (30°C) (Aswini *et al.*, 2020). BC produced by *A. senegalensis* was evaluated, maximum products detected at 37 °C of approximately (57) mg/mL wet weight and (5.21) mg/mL dry weight, discrete differences in the wet also dry weight of bacterial cellulose were found at various temperatures, for example at temperature 35°C the bacterial cellulose production reach to approximately 52 mg/mL wet weight and about 4.22 mg/mL dry weight, so these results may be lead to illation that as temperature decreases the quantity of BC produced also reduced (Aswini *et al.*, 2020). Regardless of the concentration of the substrates used in bacterial cellulose production, it observed that it able to increasing bacteria productivity, that because each M.O has an optimal temperature for growth in addition to optimal temperature for BC production that reflected on yield of BC productivity (Fernandes *etal.*, 2020).

Effect of pH

According to the results, it was observed that pH 5 gives the maximum production for bacterial cellulose AAB (5AC) isolate: the wet weight at pH 5 was (5.5gm), the dry weight was (0.26gm) and the consumed sugar was (50%). At pH 4 and pH 8 the minimum production of BC was noticed, these results could be

indicating that the BC production from acetic acid bacteria is influenced by, pH values (Figure 3).

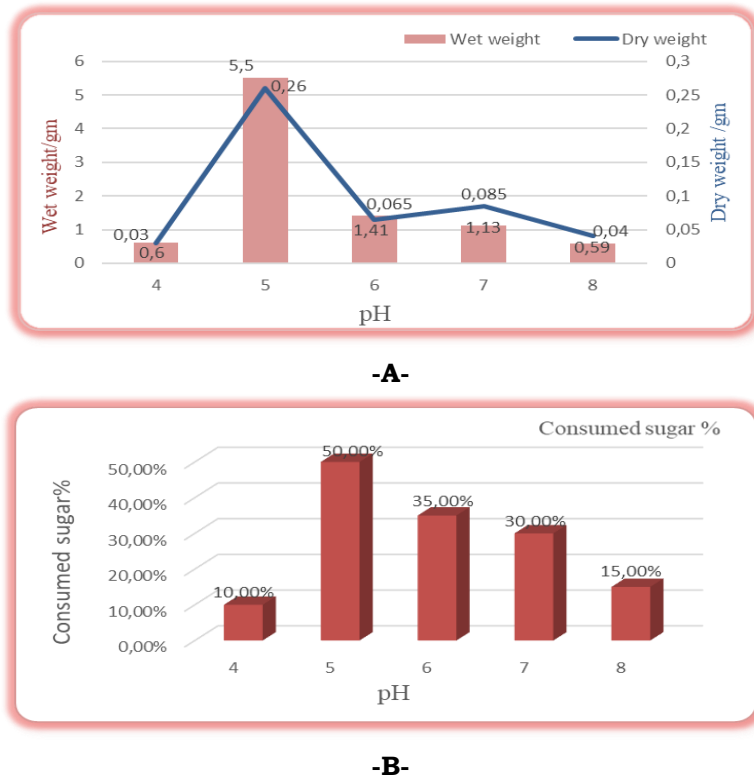


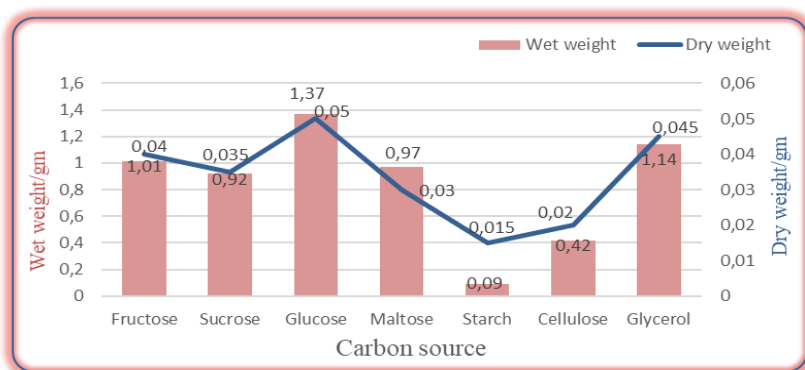
Figure (3) Effect, of pH on BNC produced by acetic acid bacteria (5AC) in HS medium, A- Wet and dry weight/gm., B- Consumed sugar (%)

The production of cellulose by *A. senegalensis* clarify that the collaboration of pH with temperature increased the yield of bacterial cellulose in Hestrin-Schramm (HS) broth to the maximum level, it was observed from experiment of Aswini *et al.* (2020) that the pH values: 2, 3, 4 and 9 showed no production of BC, the, maximum production of bacterial nano-cellulose was at the pH 4.5 that the wet weight of about 98 mg/mL and the dry weight of about 6.44 mg/mL and then followed by the pH 5 for BC production in Hestrin-Schramm broth with 85 mg/mL wet weight and dry weight about 5.48 mg /mL also could be noticed that BC produced in pH 5.5, the wet weight was about 61 mg/mL and the dry weight 4.74 mg/mL, so more increase in pH may reduce the amount of bacterial cellulose production. The medium pH is an critical parameter that may disturbing the action of primary enzymes that are responsible for of bacterial cellulose production, so this parameter must be optimized to achieve the highly concentration of bacterial cellulose (Abdul Rahman *et al.*, 2021). Prashant *et al.* (2008) observed that the activity of acetic acid bacteria *Acetobacter xylinum* to synthesize cellulose is optimum in acidic condition, the catalyst action was relative to (H⁺) concentration that further hydrogen ions formed in the medium fermentation lead to rapid production of cellulose with more quantities. Some research reported that pH in the range (4-6) could be ideal values in the

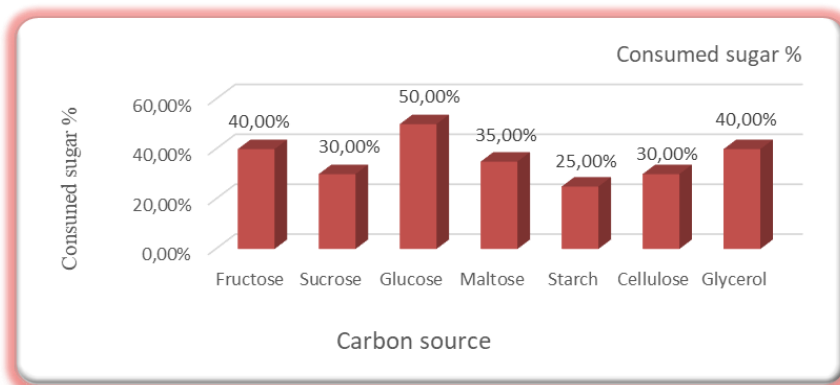
fermentation medium of bacterial cellulose and the observations of experiments indicate that: pH (5.5) required for *Acetobacter xylinum* BC production, (4.5- 7.5) needs for another strain of *A. xylinum* also pH (6.0) are required for *Komagataeibacter* spp. (Lahiri *et al.*, 2021).

Effect of different carbon sources

The HS medium containing different carbon sources was inoculated with AAB bacteria (5AC) isolate, the experiments demonstrated that the glucose (as carbon source) gives the maximum production for bacterial cellulose and the results showed that the maximum: wet weight was (1.37gm), dry weight was (0.05) and the consumed sugar was (50%), while the minimum production of BNC was at starch which observed: the wet weight (0.09gm), dry weight (0.015gm) and the sugar consumption was (25%) as noticed in Figure (4).



-A-



-B-

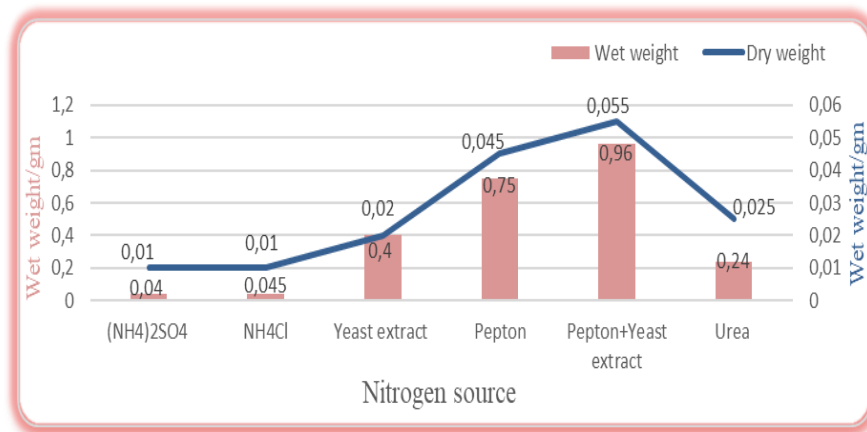
Figure (4) Effect of different C sources on BNC, production from AAB (5AC) isolate in HS medium: A- Wet and dry weight/gm., B- Consumed sugar (%)

In a research study, bacterial cellulose production by *Acetobacter* was estimated by adding 24 carbon sources, among these sources of (C), the glycerol showed maximum bacterial cellulose production, the wet weight about 248 mg/mL, and

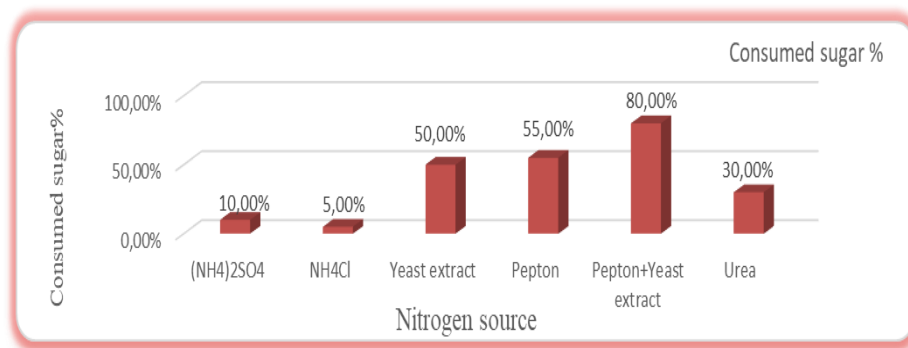
dry weight 13 mg/mL in Hestrin-Schramm (HS) broth medium and then followed by tryptose and fructose, that the produced wet weight about (114 mg/mL and 100 mg/mL) respectively, dry weight about (6.54 mg/mL and 6.17 mg/mL) BC, respectively (Aswini *et al.*, 2020). The C sources that utilized for bacterial cellulose production generally agro, industrial wastes for example rotten fruit such as pineapple peels liquid extract and sugar as a production medium (Jozala, *et al.*, 2015). The bacterial yield of cellulose production up to proximately (40%) in relative to the starting C source when BC produced in large scale (Klemm, *et al.*, 2011). When the C source used in the medium is glucose, bacterial nanocellulose biosynthesis happens in four enzymatic steps: phosphorylation of glucose, Isomerization, Synthesis of UDP- glucose and Polymerization (Ross *et al.*, 1987; Barja, 2021). Revin *et al.* (2018) showed that using the acid secondary products from dairy also from alcohol manufacturing as a source of carbon gained yields three time greater than that of the standard Hestrin-Schramm (HS) broth (2.14 g/L), also the exhausting C sources from beet molasses and whey of cheese got values larger than the standard Hestrin-Schramm medium (3.26 g/L) (Salari *et al.*, 2019). *K. xylinus* experimented for production of cellulose on different (C) sources and the greatest results determined when used the carbohydrates like Glucose, and Mannitol; the Glucose considered as highest source of carbon for bacterial cellulose production in comparison to the other sugar (Saichana *et al.*, 2015). The highest bacterial cellulose product on the base of dry weight (wt. membrane/v. medium) achieved at (2%) w/v concentration of C source, for entire sources the yield recorded (2.80, 0.380, and 1.680 g/L) of BC that using (2%) of the sugars Glucose, Fructose, and Sucrose respectively, an obvious variance detected for bacterial cellulose produced from glucose sugar, it was reported that this sugar represented as a source of energy also as a faultless precursor for polymerization of cellulose thus glucose is usually utilized as a provenance for cellulose production from many strains of acetic acid bacteria such as *Komagataeibacter* sp. (Molina-Ramírez, *et al.*, 2017).

Effect of various nitrogen sources

The production of BC requires fine balance between carbon and nitrogen sources, from the essential factors that affected growth of bacteria and production cellulose from bacteria is the nitrogen source, because the bacteria require nitrogen to complete its metabolic pathways. In this study, from the various organic and inorganic (N) sources the results proved that the (peptone, and yeast extract) together are best (N) sources for production of BNC, highest results for the produced BNC from the isolate AAB (5AC) were: wet weight (0.96gm), dry weight (0.055gm) and the consumed sugar (80%) and the lowest BNC production was at NH₄Cl: wet weight (0.045gm), dry weight (0.01) and consumed sugar (5%) and (NH₄)₂SO₄ results showed: wet weight (0.04gm), dry weight (0.01gm) and consumed sugar (10%) (Figure 5). It was concluded that the organic (N) sources are more suitable for production of bacterial cellulose from inorganic source because the organic source such as yeast extract for example is rich with: amino acids, vitamins also trace elements which encourage the bacterial multiplication and as a result could be stimulate and affects the BNC production and yield.



-A-



-B-

Figure (5) Effect of various sources of nitrogen on BNC production from AAB isolate (5AC) in HS medium: A- Wet and dry weight/gm., B- Consumed sugar (%)

The (N) sources not participate directly in BNC creation, but it's necessary for the bacterial progression and existence (Gopu and Govindan, 2018), so appropriate nitrogen supplementation may be indirectly affects BNC yield. In HS medium there was an investigation about the effect of different organic and inorganic sources of nitrogen in bacterial nanocellulose synthesis, and the usual N sources (yeast, extract and ,peptone) in Hestrin-Schramm (HS) medium replaced with another nitrogen sources, the considerable observation was that the organic N sources provided higher BNC harvests in compare to those inorganic N sources, as a result some research described that yeast, extract provided the higher yield as a particular N source when growing some species of AAB, as yet bacterial nano-cellulose was produced most powerfully in the existence of complex (N) sources (yeast, extract collective with peptone, or may be with tryptone.), in addition to this, the tests demonstrated that the complex organic nitrogen source also obligatory for *K. hansenii* (S11) for efficient biosynthesis of BNC (Cielecka *et al.*, 2021). Santo,s. *et al.* (2013) noted that *Gluconacetobacter. Sucofermentas*, synthesized bacterial nanocellulose with maximum yield only in the existence of yeast, extract and peptone, added together. As it is known the N is the main constituent of proteins it is essential in the metabolism of the cell and includes 8-

14% of bacterial dry cell mass, it was observed that single N presented significant reducing in production of bacterial cellulose, the single source of nitrogen (yeast, extract) displayed highest yield of BC than the single nitrogen $(\text{NH}_4)_2\text{SO}_4$ source which reported to decrease the growth and cellulose yields, also it was observed that adding the amino acid (methionine) for the culture of *A. xylinum*, encouraged the growth rate through the early, culture age, decreased the lag time and improved the cellulose production rate (Yodsuwan *et al.*, 2012). The nitrogen source (yeast extract) in Hestrin-Schramm (HS) broth showed maximum wet weight, of about (522 mg/,mL) yielding also (52.26 mg/mL) dry, weight of bacterial cellulose, Following the (yeast, extract), the (beef, extract) formed (105 mg/,mL) wet weight yield and (1.890 mg/,mL) dry, weight of bacterial cellulose then followed by, pepton, recording (100 mg/,mL) wet weight and (1.490 mg/,mL) dry weight. Furthermore, the production, of bacterial cellulose not reinforced via some N sources like calcium nitrat, sodium azide, ammonium nitrat,e, and urea, (Aswini *et al.*, 2020).

Conclusion

The bacterial nanocellulose (BNC) is a multipurpose biomaterial, it create applications in many processes and due to its important in various fields the refinement and fixing of the production culture condition of (BC) is important particularly in the industrial production field, in this study, the parameters: temperature at (30°C), pH of (5), glucose as source of carbon and (peptone, + yeast, extract) as N source were found as optimum conditions in (HS) broth medium to attain the maximum production of BC. The produced bacterial cellulose may be considered as very pure nano,polymer so any interferences in nano-formulation also spreading its usage toward sustainable food, medical and therapeutic sectors will be a greatly appreciable, way, forward,.

Reference

- Abdul Rahman, S.S.; Vaishnavi, T.; SaiVidyasri, G.; Sathya, K.; Priyanka, p.; Venkatachalam, P. and Karuppiyah, S. (2021). Production of bacterial cellulose using *Gluconacetobacter kombuchae* immobilized on *Lufa aegyptiaca* support. Scientific Reports. 11:2912.
- Abol-Fotouh, D.; Hassan, M.A.; Shokry, H.; Roig, A.; Azab, M.S. and Kashyout, A.B. (2020). Bacterial nanocellulose from agro-industrial wastes: low-cost and enhanced production by *Komagataeibacter saccharivorans* MD1. Scientific Reports nature research. 10:3491.
- Abouelkheir, S.S.; Kamara, M.S.; Atia, S.M.; Amer, S.A. *et al.* (2020). Novel research on nanocellulose production by a marine *Bacillus velezensis* strain SMR: a comparative study. Sci. Rep. 10, 14202.
- Aswini, K.; Gopal, N.O. and Uthandi, S. (2020). Optimized culture conditions for bacterial cellulose production by *Acetobacter senegalensis* MA1. BMC Biotechnology. 20:46.
- Barja, F. (2021). Bacterial nanocellulose production and biomedical applications. J Biomed Res. 35(4):310-317.
- Bellankimath, A.; Katti, A.; Hemalata, V.B. and Meti, B.S. (2017). Isolation and characterization of the indigenous acetic acid bacteria from Western Ghats soil samples. Int. J. Curr. Microbiol. App.Sci. 6(9): 1255-1265.

- Cacicedo, M.L.; Castro, M.C.; Servetas, I.; Bosnea, L.; Boura, K. and Tsafarakidou, P. (2016). Progress in bacterial cellulose matrices for biotechnological applications. *Bioresour Technol.* 213:172–80.
- Cielecka, I.; Rynagajłło, M.; Maniukiewicz, W. and Bielecki, S. (2021). Highly Stretchable Bacterial Cellulose Produced by *Komagataeibacter hansenii* SI1. *Polymers.* 13(24): 4455.
- Costa, A.F.S.; Almeida, F.C.G.; Vinhas, G.M. and Sarubbo, L.A. (2017). Production of bacterial cellulose by *Gluconacetobacter hansenii* using corn steep liquor as nutrient sources. *Frontiers in microbiology.* 8:2027.
- Donini, I.A.N.; De Salvi, D.T.B.; Fukumoto, F.K.; Lustri, W.R.; Barud, H.S. and Marchetto, R. (2010). Biosynthesis and recent advances in production of bacterial cellulose. *Eclat Quim.* 35:165-78.
- Fernandes, I.A.A.; Pedro, A.C.; Ribeiro, V.R.; Bortolini, D.G.; Ozaki, M.S.C.; Maciel, G.M. and Haminiuk, C.W.I. (2020). Bacterial cellulose: From production optimization to new applications. *Int J Biol Macromol.* 164: 2598-2611.
- Goldberg, R.N.; Schliesser, J.; Mittal, A.; Decker, S.R.; Santos, A.F.; LO, M.; Freitas, V.L.S.; Urbas, A.; Lang, B.E. and Heiss, C. (2015). A thermodynamic investigation of the cellulose allomorphs: Cellulose (am), cellulose I β (cr), cellulose II(cr), and cellulose III(cr). *J. Chem. Thermodyn.* 81: 184–226.
- Gomes, R.J.; Borges, M.F.; Morsyleide de Freitas Rosa, M.F.; Jorge, R.; Castro-Gómez, H. and Spinosa, W.A. (2018). Acetic Acid Bacteria in the Food Industry: Systematics, Characteristics and Applications. *Food Technology and Biotechnology* 56 (2).
- Gopu, G. and Govindan, S. (2018). Production of bacterial cellulose from *Komagataeibacter saccharivorans* strain BC1 isolated from rotten green grapes. *Prep. Biochem. Biotechnol.* 48(9): 842-852.
- Holt, J.M.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.Y & Williams, S.T. (1994). Genus *Acetobacter* and *Gluconobaceter*. In Bergery's Manual of Determinative Bacteriology. 9th edn., Williams & Ilkens, Maryland, U.S.A. pp: 71-84.
- Iguchi, M.; Yamanaka, S. and Budhiono, A. (2000). Bacterial cellulose —a masterpiece of nature's arts. *J Mater Sci.* 35(2): 261–270.
- Jozala, A.; Pe´rtile, R.; dos Santos, C.; de Carvalho Santos-Ebinuma, V.; Seckler, M.; Gama, F. and Pessoa, A.J. (2015). Bacterial cellulose production by *Gluconacetobacter xylinus* by employing alternative culture media. *Appl Microbiol Biot.* 99:1181–1190.
- Klemm, D.; Kramer, F.; Moritz, S.; Lindstro“m, T.; Ankerfors, M.; Gray, D. and Dorris, A. (2011). Nanocelluloses: a new family of nature-based materials. *Angew Chem Int Edit.* 50:5438– 5466.
- Lahiri, D.; Nag, M.; Dutta, B.; Dey, A.; Sarkar, T.; Pati, S.; Edinur, H.A.; Abdul Kari, Z.; Mohd Noor, N.H. and Ray, R.R. (2021). Bacterial Cellulose: Production, Characterization, and Application as Antimicrobial Agent. *Int. J. Mol. Sci.* 22, 12984. <https://doi.org/10.3390/ijms222312984>.
- Lee, K.Y.; Tammelin, T.; Kiiskinen, H.; Samela, J.; Schlufte, K. and Bismarck, A. (2012). Nano-fibrillated cellulose vs bacterial cellulose: reinforcing ability of nanocellulose obtained topdown or bottom-up. ECCM15-15th European conference on composite materials, venice, Italy.
- Molina-Ramírez, C.; Castro, M.; Osorio, M.; Torres-Taborda, M.; Gómez, B.; Zuluaga, R.; Gómez, C.; Gañán, P.; Rojas, O. J. and Castro, C. (2017). Effect of different carbon sources on bacterial nanocellulose production and structure

- using the low ph resistant strain *Komagataeibacter medellinensis*. *Materials* (Basel, Switzerland). 10(6): 639.
- Prashant, R., Bajaj, I.B., Shrikant, A. S. and Rekha, S. S. (2008). Fermentative production of microbial cellulose. *Food technology biotechnol.* (2): 107-124.
- Ramli, R. M., Sinrang, A. W., & Aminuddin. (2021). Levels of alpha-1 acid glycoprotein (AGP) in stunting and non stunting tolls age 36-60 months . *International Journal of Health & Medical Sciences*, 4(1), 145-149. <https://doi.org/10.31295/ijhms.v4n1.1666>
- Revin, V.; Liyaskina, E.; Nazarkina, M.; Bogatyreva, A. and M. Shchankin, M. (2018). Cost-effective production of bacterial cellulose using acidic food industry by-products. *Brazilian J. Microbiol.* 49: 151-159.
- Ross, P.; Mayer, R. and Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiol. Rev.* 55 (1): 35-58.
- Ross, P.; Mayer, R. and Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiol. Rev.* 55 (1): 35-58.
- Ross, P.; Weinhouse, H. and Aloni, Y. (1987). Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. *Nature.* 325(6101): 279-281.
- Saichana, N.; Matsushita, K.; Adachi, O.; Frébort, I. and Frebortova, J. (2015). Acetic acid bacteria: A group of bacteria with versatile biotechnological applications. *Biotechnol. Adv.* 33: 1260-1271.
- Salari, M.; Khiabani, M.S.; Mokarram, R.R.; Ghanbarzadeh, B. and Kafil, H.S. (2019). Preparation and characterization of cellulose nanocrystals from bacterial cellulose produced in sugar beet molasses and cheese whey media, *Int. J. Biol. Macromol.* 122: 280-288.
- Santos, S.M.; Carbajo, J.M.; Villar, J.C. (2013). The Effect of Carbon and Nitrogen Sources on Bacterial Cellulose Production and Properties from *Gluconacetobacter sucrofermentans* CECT 7291 Focused on Its Use in Degraded Paper Restoration. *BioResources.* 8(3): 3630-3645.
- Sathar, J. (2012). Optimization of bacterial cellulose production from *Acetobacter xylinum* by using response surface methodology (Rsm). Thesis of the degree of Bachelor of Chemical Engineering (Biotechnology). University Malaysia Pahang.
- Sijabat, E.K.; Nuruddin, A.; Aditiawati, P. and Purwasasmita, B.S. (2020). Optimization on the synthesis of bacterial nano cellulose (BNC) from banana peel waste for water filter membrane applications. *Materials Research Express.* 7(5): 055010.
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, 5(1), i-v. <https://doi.org/10.53730/ijhs.v5n1.2864>
- Teoh, A.L.; Heard, G. and Cox, J. (2004). Yeast ecology of Kombucha fermentation. *Int J Food Microbiol.* 95:119-126.
- Thongwai, N.; Futui, W.; Ladpala, N.; Sirichai, B.; Weechan, A.; Kanklai, J. and Rungsirivanich, P. (2022). Characterization of Bacterial Cellulose Produced by *Komagataeibacter maltaceti* P285 Isolated from Contaminated Honey Wine. *Microorganisms.* 10, 528.
- Trinh, N.T.N.; Masniyom, P. and Maneesri, J. (2016). Optimization of culture condition for *Acetobacter aceti* TISTR 102 in coconut water with supplementary banana juice. *International Food Research Journal.* 23(3): 1300-1307.
- Yodsuwan, N.; Owatworakit, A.; Ngaokla, A.; Tawichai, N. and Soykeabkaew, N. (2012). Effect of carbon and nitrogen sources on bacterial cellulose production for bionanocomposite materials. Conference Paper. Research Gate. The 1st

- MFUIC 2012 At: Mae Fah Luang University, Chiang Rai, Thailand.
<https://www.researchgate.net/publication/280730054>.
- Zielińska, S.; Matkowski, A.; Dydak, K.; Czerwińska, M.E.; Dziągwa-Becker, M.; Kucharski, M.; Wójciak, M.; Sowa, I.; Plińska, S.; Fijałkowski, K. and Gorczyca, D. (2022). Bacterial Nanocellulose Fortified with Antimicrobial and Anti-Inflammatory Natural Products from *Chelidonium majus* Plant Cell Cultures. *Materials*. 15, 16.
- Zuppolini, S.; Salama, A.; Cruz-Maya, I.; Guarino, V. and Borriello, A. (2022). Cellulose Amphiphilic Materials: Chemistry, Process and Applications. *Pharmaceutics*. 14, 386.