JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(10), 318–330
https://doi.org/10.12911/22998993/192037
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.07.13
Accepted: 2024.08.21
Published: 2024.09.01

Reuse of Date Fruit Processing Waste as Substrate for Biological Nanocellulose Production – A Sustainable Disposal Approach

Husam Al-Hamaiedh[1,2], Omar A. Abdulateef[2], Laith M. Najeeb[3], Khawla D. Al-Hamaideh[4*]

[1] Civil Engineering Department, Faculty of Engineering Technology, Zarqa University, 2000 Zarqa 13110, Jordan

[2] Civil and Environmental Engineering Department, Faculty of Engineering, Mutah University, Al-Karak 61710, Jordan

[3] College of Science, Anbar University, 55431 Baghdad, 55 Ramadi, Ramadi, 31001, Iraq

[4] Department of Basic Medical Sciences, Faculty of Medicine, Al-Balqa Applied University Al-Salt, Jordan

* Corresponding author's e-mail: husamh@mutah.edu.jo

ABSTRACT

Date pomace (DP), the primary source of the solid wastes generated from date processing industry, characterized by high sugar concentration and elevated moisture content, it constitutes about 30% of the weight of the processed dates. In addition to the safe disposal, incorporating DP in the medium for bacterial nanocellulose (BNC) production as nutrient source can significantly reduce the production cost. This study aims to investigate the influence of the operational conditions namely the initial pH, incubation temperature, and DP juice ratio in the medium on the yield, water holding capacity, moisture content and morphology of the produced BNC. samples with constant initial pH, and fixed juice ratio were incubated at different temperatures. To explore the optimal initial pH value samples with fixed juice ratio were incubated at 30 °C. Samples with different juic ratio and fixed initial pH value were incubated at 30 °C. The structure and morphology of the produced BNC were tested using Field Emission Scanning Electron Microscopy (FESEM). The Maximum BNC wet and dry yields of 31.6 g/L and 0.62 g/L were achieved at an initial pH of 6, an incubation temperature of 30 °C, and a juice ratio in the medium of 10%, respectively. The results FESEM revealed that doubling the DP juice ratio in the culture medium increased the porosity and regularity of the porous structure of the produced BNC. Increasing juice ratio in the medium decreased the yield, water holding capacity and moisture content of the produced BNC. Since oxygen is developed throughout the fermentation process and different types of sugars are produced from the degradation of starch elements in DP, it was difficult to establish a correlation between the sugar and oxygen consumption and the resultant BNC yield. The findings validated the feasibility of employing DP juice as a substrate for the manufacture of nanocellulose material with the added benefit of mitigating environmental pollution.

Keywords: nanocellulose, date, pomace, yield, fermentation, bacteria, waste.

INTRODUCTION

Palm dates have become an essential part of the diet in a number of nations located in the Middle East and North Africa because of their exceptionally high nutritional content. Dates include a wide variety of essential components, including carbohydrates, vitamins, proteins, minerals, and lipids [Siddiq et al., 2013]. More than 2.000 varieties of dates, each with its own particular physical and chemical characteristics, have been identified across the globe [Alkaabi et al., 2011]. Approximately 12.52 million tons of date fruit were produced worldwide, according to the report published by the FAOSTAT [2023]. The countries of Egypt, Saudi Arabia, Iran, Algeria, Iraq, Pakistan, Sudan, Oman, the United Arab Emirates, and Tunisia were the top 10 producers of palm data according to statistics published by FAOSTAT [2023]. A significant part of the collected date

fruit is used in the process of extracting date juice (date molasses), vinegar, jelly, jam, powder, and other products [Barreveld, 1993]. Date processing industry are responsible for the production of huge amount of date residues such as date seeds, date press cake (DPC) or date pomace (DP), and cull dates (out grade dates), which are either used as animal feed or end up in drains and dumps. Date residues can be found in a variety of forms [Alsafadi et al., 2020]. According to Plazzotta et al. [2017], the wastes generated from date processing have a high concentration of biodegradable organics and a high moisture content, which allow leachate to be produced and aromas to spread throughout the surrounding environment. Furthermore, the disposal of untreated date processing waste into the environment promotes the growth of germs, pests, and mice, which in turn facilitates the spread of diseases [Heok et al., 2018]. The date pomace DP is the main component of the solid waste produced in date industry. It makes up around 30% of the processed dates' weight and is distinguished by having a high sugar and moisture content [Barreveld, 1993]. Many studies have been conducted on material and energy recovery of solid waste as secondary raw materials for industry. The organic fraction of municipal solid waste has been reused for compost production [Nawaeseh et al., 2012]. Biogas production through the co digestion of sewage sludge and MSW as well as from the organic fraction of MSW has achieved [Aljbour et al., 2021; Al-Hajaya et al., 2021]. A successful reuse of marble and granite sludge as replacements for raw materials in the ceramic industry and as fine aggregate in mortar and concrete was acheived [Al-Hamaiedh, 2010; Al-Hamaiedeh and Khushefati, 2013; Al-Jarajreh, et al., 2023]. Al-Awabdeh et al. [2022] explored the partial replacement of fine aggregate by glass waste. Additionally, successful incorporation of face masks in concrete mixtures was achieved by Al Swalqah et al. [2023]. DP has been used as a raw material in the manufacturing of many products, including fertilizers [Al-Farsi et al., 2007; Rambabu et al., 2020] activated carbon [Hasanzadeh et al., 2020; Heidarinejad et al., 2018] and nanoparticles for the treatment of wastewater [Agarwal et al., 2017; Majzoobi et al., 2019; Norouzi et al., 2018].

The high content of protein, sugar, and dietary fiber in DP makes it an ideal carbon source for the production of BNC which is a natural biopolymer produced by particular bacterial species [Majzoobi et al., 2019; Gullo et al., 2017]. In general, nanocellulose (NC) has micrometer-scale length variations with diameters ranging from 5 to 60 nanometers [Carpenter, 2015]. Practically, the NC may be produced in two ways, either from vegetable cellulose using a number of chemical and mechanical processes, or it can be produced by aerobic fermentation using certain acid bacteria to produce the BNC. The synthesis of BNC is on the rise due to its mechanical, and optical qualities and low environmental impact. The produced BNCs are used in a wide range of applications, such as drug delivery systems, tissue regeneration, in vitro and in vivo tissue engineering, and vascular grafts [Azeredo, 2013; Czaja et al., 2007; Almeida et al., 2014]. Because of its versatile properties, BNC has been the subject of extensive study using various substrates such as beet molasses, black tea broth, and date molasses in an effort to find inexpensive and easily accessible substrates that lower the production costs and increase the volume of production [Kiziltas et al., 2015b, Goh et al., 2012, Al-Maleki and AL-Shamary, 2013]. Most of the researches are directed toward improving and upscaling the production of BNC through microbial hosts. Early studies used a medium with 2% glucose (the main carbon source), 0.5% peptone, 0.5% yeast extract, 0.27% anhydrous disodium phosphate, and 0.15% citric acid monohydrate as the standard culture media for the synthesis of BNC [Schramm and Hestrin 1954]. The primary determinants of the yield and qualities of the generated BNC are the incubation temperature, pH, and oxygen availability in addition to the substrate type and ratio in the medium. This study aims to investigate the influence of the operational conditions (initial pH, incubation temperature, and DP juice ratio in the medium) on the yield, water holding capacity, moisture content and morphology of the produced BNC.

MATERIAL AND METHODS

Figure 1 shows the summarized graphical research methodology.

Experimental work setup

Samples of DP were collected from Hit-factory – in Iraq, where molasses and vinegar produced from the collected dates "Zahdi" type. The extraction of juice from DP was performed following the method described by Jozala et al.

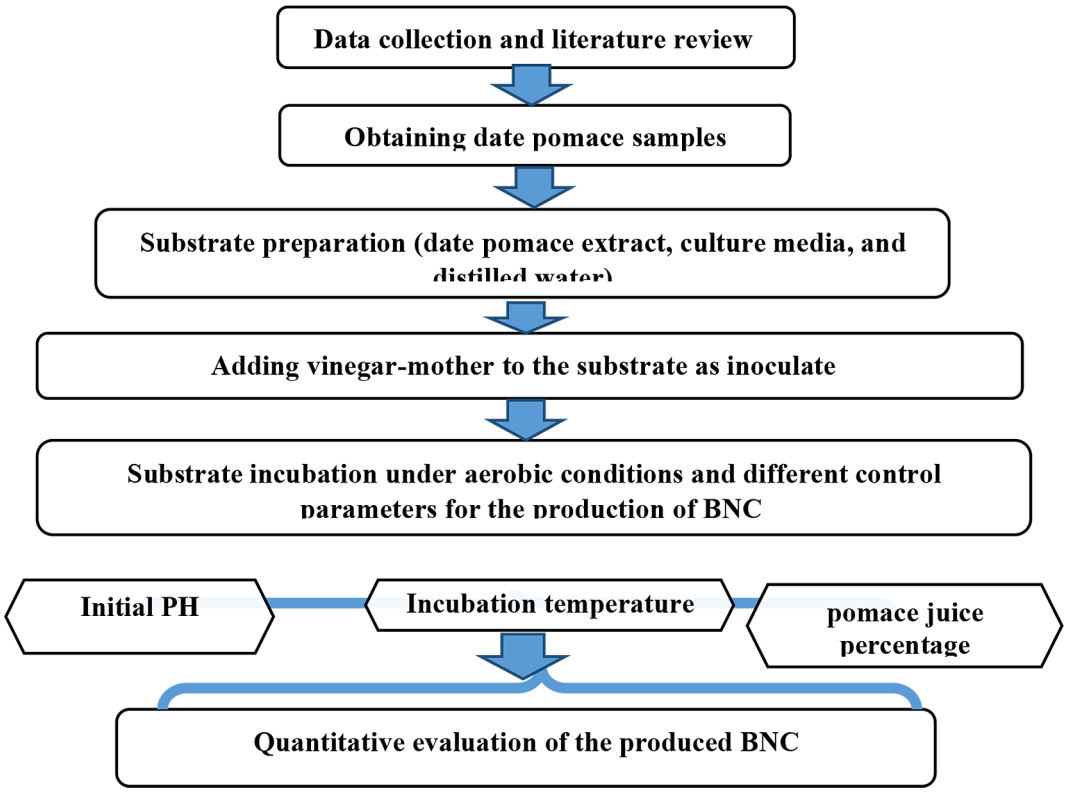

Figure 1. Research methodology outlines

[2015] with minor amendments. A 500 g of DP was soaked in 800 ml of distilled water for 30 minutes and then mixed in a blender, the mixture had been filtered through a fine cotton texture media. The filtrate was processed by a centrifuge at 4000 rpm for 20 minutes to extract the juice, the extracted supernatant (juice) was collected and sterilized in autoclave at 121 °C for 30 min then stored at 4 °C. The DP before and after juice extraction as well as the extracted juice is shown in Figure 2. Modified Hestrin-Schramm (HS) culture medium was used in the process of BNC production. Standard HS medium consists of glucose (20) g/L, peptone (5 g/L), yeast extract (5 g/L), sodium di-basic hydrogen phosphate (2.7 g/L), and citric acid (1.15 g/L). The modified HS was prepared following the method described by Abol-Fotouh et al. [2020] with replacing glucose with date pomace juice.

One liter beakers were used with only half the volume used in order to provide the largest average surface area: container depth. Experiments P1–P7 were carried out with control of the main determinants affecting the growth of bacteria.

Mother of Vinegar was used in Inoculating which was obtained from the same factory to ensure that they contain commensal acid bacteria varieties that are resistant to the same conditions of date pomace. Mother of Vinegar is a gelatinous layer consisting of several classes of acid bacteria that colonize a form of cellulose produced by these bacteria, and this layer is produced when sugary liquids are fermented as in production of vinegar. Vinegar mother is similar to kombucha (Figure 3) which represents a symbiotic culture of one or more species of bacteria and yeasts 'scoby' within a cellulose tissue. Proceeding from the great similarity between mother of vinegar and kombucha, mother of vinegar was used to inoculate the brood in the manner mentioned in Goh et al. [2012]. Fermentation was carried out by using 3% of vinegar mother to inoculate culture medium (weight/volume on wet weight basis).

The medium was prepared by adding the weighted medium components using an accurate balance and then adding them to the distilled water with continuous stirring until dissolution. PH was adjusted using HCI acid or NaOH base as needed. Then the beakers were autoclaved at 121°C for 20 minutes then adding inoculation inside Laboratory Hood. Then they were statically incubated in dark incubator with normal aeration at deferent parameters for 168 ± 1 hour, the resulting cellulose layer

Figure 2. Date pomace (a) before extracting, (b) after extracting and date pomace extract "pomace juice"

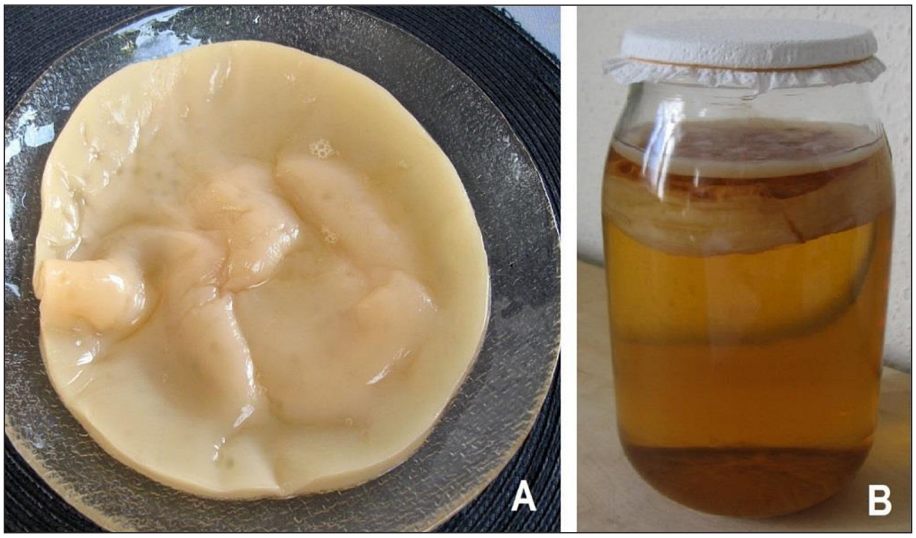

Figure 3. (a) Mother of vinegar, (b) Kombucha scoby

on the surface of the media was harvested and physical analyses were performed on it.

The effect of the control parameters on bacterial growth, and hence the yield and properties of the produced BNC, was investigated in seven experimental sets, P1–P7 (Table 1). The initial pH level, DP juice ratio in the substrate, and the incubation temperature were the studied control parameters. Each experiment in the set was carried out in a 1-liter beaker with 500 ml of substrate that included 3% mother of vinegar (inoculate culture), date juice extract, and the culture media HS [Goh et al., 2012]. During the preparation of the medium, the constituents of the medium were weighed using an accurate analytical balance with an accuracy of 0.001 g. The quantity of distilled water required was measured out using a graduated cylinder. Before and after the incubation period, the initial and final pH and oxygen levels were measured, respectively, using a pH meter and an oxygen meter. The pH was adjusted with HCI and NaOH to the proper level before to introducing the inoculum, and the samples were sterilized in an autoclave at 121 °C for 20 minutes. The samples were incubated in a dark, open space for 168 hours at the predetermined temperature. After harvesting the cellulose layer Figure 3, that had formed on the surface of the medium following incubation, measurements were made of the wet and dry yield, water holding capacity, and moisture content of the produced BNC. Table 1 summarizes the conditions under which the experiments were conducted. Samples from sets P1, P3, P6, and P7, with a fixed pH of 6 and a DP juice ratio in the medium of 10%, were incubated at different temperatures (24 °C, 26 °C, 30 °C, and 34 °C) to explore the optimal incubation temperature.

Additionally, samples from sets P2, P3, and P4, with pH values of 5, 6, and 7 respectively, and a fixed DP ratio of 10%, were incubated at

Table 1. Constituents and conditions of the conducted experiments for BNC production

Experimental set	Total media (ml)	Modified HS percentage (%)	Juice percentage (%)	Initial PH (before sterilization ± 0.1)	Incubation temperature (°C ± 0.5)	Inoculator percentage (mother of vinegar) (weight/volume) (%)	Inoculator weight (mother of vinegar) (g ± 1g)
P1	500	90%	10%	6	26	3%	15
P2	500	90%	10%	5	30	3%	15
P3	500	90%	10%	6	30	3%	15
P4	500	90%	10%	7	30	3%	15
P5	500	80%	20%	6	30	3%	15
P6	500	90%	10%	6	34	3%	15
P7	500	90%	10%	6	24	3%	15

30 °C to determine the optimal pH value. Furthermore, samples from sets P3 and P5, with DP ratios of 10% and 20% respectively, were incubated at a constant pH of 6 and a temperature of 30 °C to determine the optimal juice ratio within the medium. Moreover, the concentrations of the primary sugars before and after the fermentation process were evaluated in order to study the impact of the control parameters on sugar consumption for BNC production. Using a kit designed for each variety of sugar, the colorimetric method of determining sugar content was applied. Equation 1 was used to determine the ratios of fructose and glucose consumption.

$$Consumption\ ratio = (I.S.C. - R.S.C.) / I.S.C. \quad (1)$$

where: *I.S.C.* – initial sugar concentration (mg/ml), *R.S.C.* – remaining sugar concentration (mg/ml).

The harvested cellulose layer contained different residual medium components, Figure 4 which were removed by washing the BNC membranes twice with boiling distilled water and 0.1 N NaOH for a duration of 20 minutes for each step. The final step involved washing the BNC with distilled water until reaching a neutral pH value Figure 4. The wet and dry weights as well as water holding capacity of BNC have been measured following the methods mentioned in (Abol-Fotouh et al., [2020]. The film layer was kept in water at 4 °C and weighed to get the wet weight, while the dry weight of the film was measured after drying at room temperature for 24 hours. The water holding capacity (WHC) was calculated using equations 2 and the moisture content ratio of BNC was calculated using Equations 3 and 4 which correspond to the wet mass and the dry mass methods, respectively:

$$WHC = wet\ weight / dry\ weight \quad (2)$$

$$Moisture\ content\ ratio\ (wet\ mass\ method) = (wet\ weight - dry\ weight) / wet\ weight \quad (3)$$

$$Moisture\ content\ ratio\ (dry\ mass\ method) = (wet\ weight - dry\ weight) / dry\ weight \quad (4)$$

The structure and morphology of the harvested BNC was tested using FESEM.

Figure 4. Formed BNC-film on culture media after incubation time

RESULTS AND DISCUSSIONS

BNC yield

The effect of incubation temperature

According to the results shown in Figure 5, the optimal incubation temperature is 30 °C, which agrees with the findings published by Volova et al. [2018]. It is evident that further increase above 30 °C or decreases below 28 °C, cause sharp fall in bacterial growth subsequently in BNC production. Although the physiological temperature range is a wide 20–37 °C, it appears that bacterial growth and BNC synthesis could occur at room temperature, between 28 and 30 °C. Mesophilic temperatures produced the optimal conditions for bacterial growth and BNC production, suggesting that temperature had a significant influence on the results achieved [Khleifat, 2006]. Alternatively, this could simply be the result of temperature effects on enzyme activity [Khleifat, 2007]. According to certain reports, temperature may affect the breakdown of various organic molecules in an equal or greater way than nutrition availability [Khleifat, 2007; Khleifat et al., 2015].

The impact of initial pH

The results show that the highest yield had been achieved in samples from set P3 where the initial pH was 6, Figure 6. This took place due to the fact

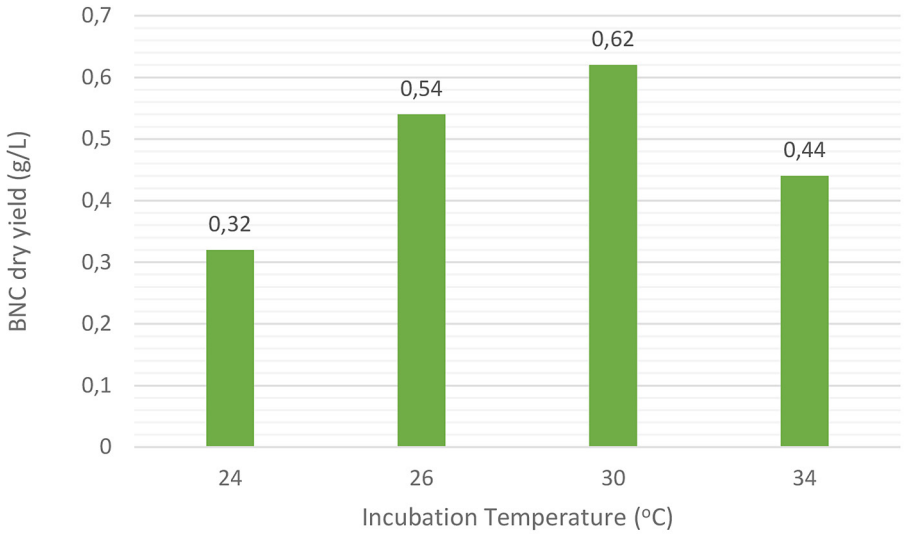

Figure 5. Effect of incubation temperature on the dry yield of BNC

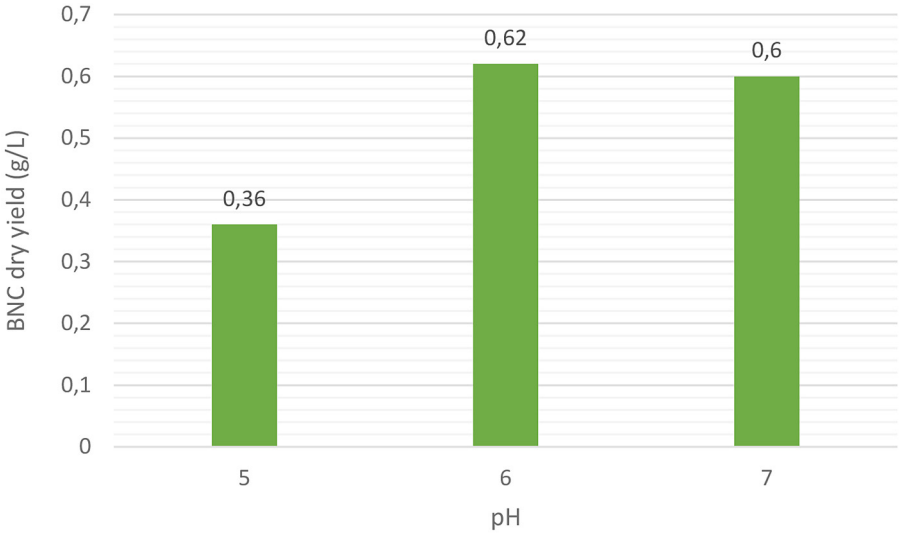

Figure 6. The effect of initial pH on the dry yield of the produced BNC

that the enzymes involved in BNC production reaching their peak activity at pH 6. These results align with those found by Son et al. [2001] which demonstrate that the maximum cellulose yield was attained at an initial pH value of 6.5. According to the conclusions of study [Delmer and Amor, 1995] the optimal pH level range at which acidic bacteria can thrive is between 4.0 and 7.0. Additionally, Alva and Peyton [2003] reported that the optimal pH for the bioremediation of organic residues differed among bacteria. For instance, the pH range for the bacterium *Halomonas campisalis* has been determined to be between 8 and 11 and the pH for the bioremediation of phenol by *Klebsiella oxytoca* was 6.8 [Khleifat et al., 2015]. The data shown in Figure 7 presents the change in the medium final pH values, which reveal that a drop in the final pH values in all samples had took place. The decrease in final pH values was due to the formation of acids during the fermentation process. Moreover, the release of carbon dioxide and creation of gluconic acid led to the formation of carbonic acid causing further drop in the medium's final pH value. These results are in consistence with the results obtained by Abol-Fotouh et al. [2020], they concluded that the production of BNC can be significantly enhanced by optimizing the medium's final pH value and sugar concentration. The wet yields of BNC produced from all samples from P1 to P7 are displayed in Figure 8.

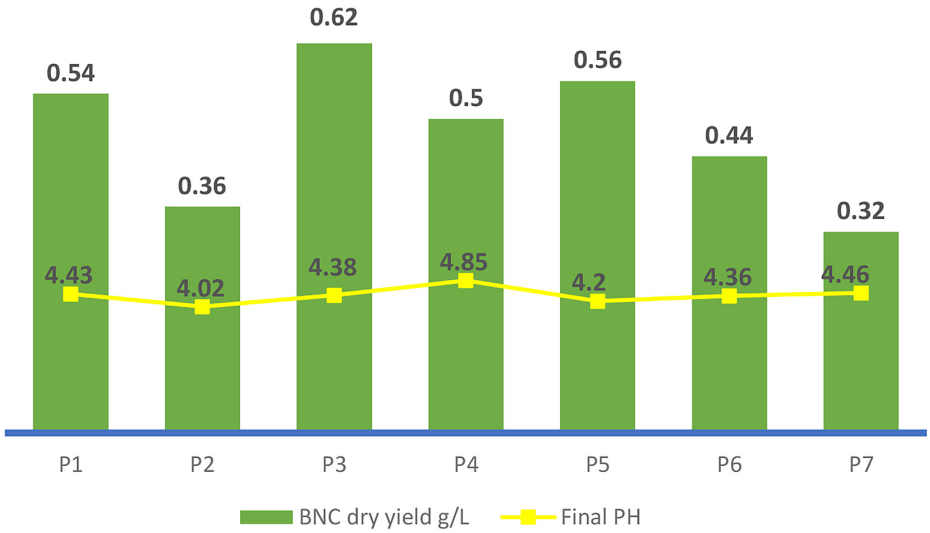

Figure 7. The relationship between BNC yield and the final pH value

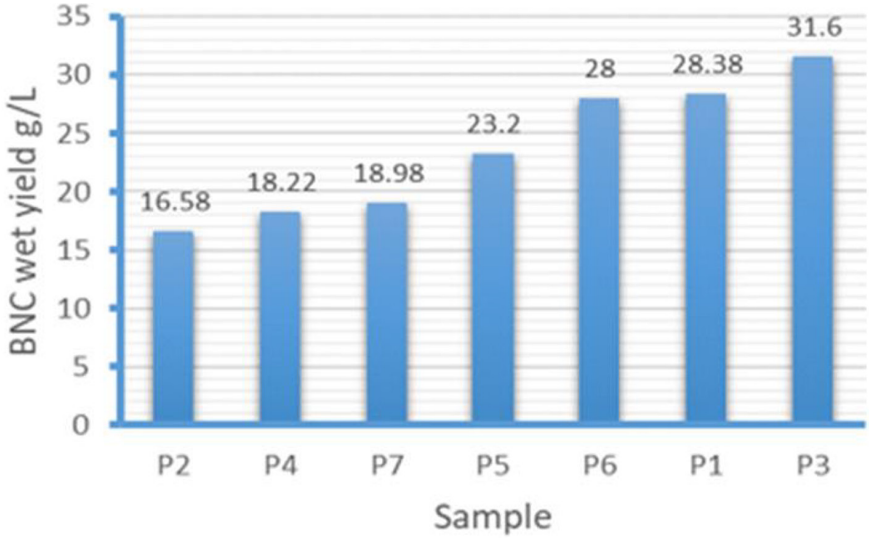

Figure 8. The wet yields of BNC from all samples

The impact of juice ratio on BNC yield

Samples from sets P3 and P5 with juice ratios of 10% and 20% respectively, have the same initial pH of 6 were incubated at temperatures of 30 °C. The dry and wet yields of BNC produced in samples from P3 having juice ratio of 10% were 0.62 g/L and 31.6 g/L while the yields produced in samples from P5 having juice ratio of 20% were 0.56 and 23.2 g/l (Figures 7 and 8). The reduction in the yield may be attributed to high initial fructose contents in samples with 20% juice ratio, which contributed to the sample's low consumption ratios. Although the cell biomass grew, the BNC yield production was inhibited at 20% glucose and fructose utilization; this could be due to catabolite repression by glucose and fructose or other carbon sources produced during fermentation [Khleifat, 2006].

The change in dissolved oxygen concentration

The fermentation process is aerobic, which cause a decrease in DO concentration in the culture medium over time. On the other hand, the culture medium receives DO from two sources, the first one is the liberated DO from the consumption of carbon from sugars and the second is DO from the air through the culture surface area in contact with air [Schramm and Hestrin, 1954]. Therefore, the DO concentration is affected by the contact surface area and the activity of the bacteria that consumes oxygen directly and that releases DO during the fermentation of sugars. As a result, the change in DO concentration is variable and does not follow a specific trend, as shown in Table 2.

Effect of sugar consumption on BNC yield

It was possible to determine the impact of sugar consumption on the behavior of the bacterial community that produces nanocellulose under various conditions by comparing the sugar concentrations in the DP juice before and after the fermentation process. Prior to fermentation, the juice had glucose and fructose concentrations of 445.22 mg/ml and 970.76 mg/ml, respectively. In samples P1, P2, P3, P4, P6, and P7, where the juice ratio was 10%, the remaining concentrations of glucose after fermentation were progressively 21.85, 29.96, 28.24, 24.02, 25.98, and 25.87 mg/ml, while the concentrations of fructose were correspondingly 55.02, 32.57, 50.24, 42.82, 21.18, and 37.87 mg/ml. In sample 5, which contained 20% juice, the quantities of glucose and fructose have been determined to be 73.56 mg/ml and 116.48 mg/ml, respectively Table 3. During the fermentation process, the starch components in DP degraded, producing a variety of monosaccharides, disaccharides, and polysaccharides. Therefore, it is difficult to create a precise correlation between the ingested Fructose and Glucose and the generated dry yield of BNC because the products of starch components degradation affect the consumption ratios of fructose and glucose [Medic, 2011].

Increasing the rate of glucose conversion while keeping a particular biomass is the most important element in determining the outcome of BNC fermentation [Bamigbade et al., 2022]. To obtain sufficient biomass, it has been reported that D-mannitol or D-sorbitol and yeast extract are typically included in the seed medium on which bacteria are inoculated, while D-sorbitol and a fairly high concentration of glucose are required in the fermentation medium [Bamigbade et al., 2022].

According to [Loh and Wang, 1998], residual glucose or fructose must be removed after trans-glycosylation due to their inhibiting effects, which therefore leads to a reduced BNC yield. As a result, fructose, yeast extract, and casein could be converted into readily mobilized intracellular carbon, nitrogen, and energy sources that could give the strain a selective advantage [Leonard and

Table 2. The change in the dissolved oxygen in the culture media

Experiment (pattern)	DO Before Incubation (mg/L)	DO After Incubation (mg/L)	Dissolved-oxygen decrease (mg/L)
P1	6.9	1.2	5.7
P2	6.3	5.2	1.1
P3	6.9	2.8	4.1
P4	6.4	4.3	2.1
P5	6.2	4.8	1.4
P6	7.2	0.4	6.8
P7	6.9	1.4	5.5

Table 3. The relation between sugar consumption ratios and dry yield of pure Cellulose at different control parameters

Sample	Initial pH	Incubation temperature, °C	Dry yield of pure cellulose (mg/L)	Initial fructose concentration (mg/ml)	Remaining fructose (mg/ml)	Fructose consumption ratio	Initial glucose (mg/ml)	Remaining glucose (mg/ml)	Glucose consumption ratio	FCR/GCR
P1	6	26	0.54	97.07	55.02	43.3%	44.52	21.85	50.9%	0.85
P2	5	30	0.36	97.07	32.57	66.4%	44.52	29.96	32.7%	2.03
P3	6	30	0.62	97.07	50.24	48.2%	44.52	28.24	36.6%	1.32
P4	7	30	0.5	97.07	42.82	55.9%	44.52	24.02	46.0%	1.22
P5	6	30	0.56	194.15	116.48	40.0%	89.04	73.56	17.4%	2.30
P6	6	34	0.44	97.07	21.18	78.2%	44.52	25.98	41.6%	1.88
P7	6	24	0.32	97.07	37.87	61.0%	44.52	25.87	41.9%	1.46

Lindly, 1999]. These substrates could be kept at a specific level to prevent completely suppressing the formation of BNC yield and to maintain cellular growth potential. In general, increasing cell biomass and using more easily metabolizable carbon and nitrogen sources result in the induction of the BNC yield formation when these carbon and nitrogen sources are added.

Water holding capacity (WHC)

Nanocellulose is characterized by its high ability to hold water, which made it effective in many applications. The high porosity and large surface area relative to mass are the main reasons behind the ability of BNC to hold large amounts of water. The produced BNC samples showed a clear difference in terms of their ability to hold water, due to the variation in their porosity and surface area which depends on the operational parameters such namely initial pH, incubation temperature, and juice concentration under which they produced as shown in Table 4.

FESEM

The morphological images of the produced BNC are shown in Figures 3 and 4, indicate the presence of structures similar to nano porous or Sponge-like nanostructure with different nano porous size. The BNC structure formed in sample P2, characterized by a high initial pH value, exhibits greater compactness and higher porosity when compared to those produced in samples P3 and P4, which had lower initial pH under identical conditions.

As for sample (P5), which contained higher DP juice ratio, the results indicate the presence of nanostructures similar to Nano fibers with very small diameters, images P5 and P5-1. Here the nano porous can be consisting of an inorganic and organic framework supporting a regular, porous structure, and it can be classified as membranes and bulk materials. Moreover, the size of the pores is generally 100 nm or smaller. Depending on this, the high ratio of juice in the medium affects the surface morphology of the produced BNC. The effect of raising the incubation temperature was positive on the alignment of the nanostructure and porosity in sample P6 compared to samples P1 and P3 which have the same pH and DP juice ratio. The results of FESEM revealed that porous nanostructures were obtained with different nanoscale diameters less than 100 nm and different porosity due to the difference in the

Table 4. Water holding capacity and moisture content of BNC samples

Pattern	Wet weight of cellulose film (g)	The dry weight of cellulose film (g)	WHC (g/g)	Moisture content ratio (wet mass method)	Moisture content ratio (dry mass method)
P1	14.19	0.27	52.56	98.1%	5155.6%
P2	8.29	0.18	46.1	97.8%	4505.6%
P3	15.8	0.31	50.97	98.0%	4996.8%
P4	9.11	0.25	36.44	97.3%	3544.0%
P5	11.6	0.28	41.43	97.6%	4042.9%
P6	14	0.22	63.64	98.4%	6263.6%
P7	9.49	0.16	59.31	98.3%	5831.3%

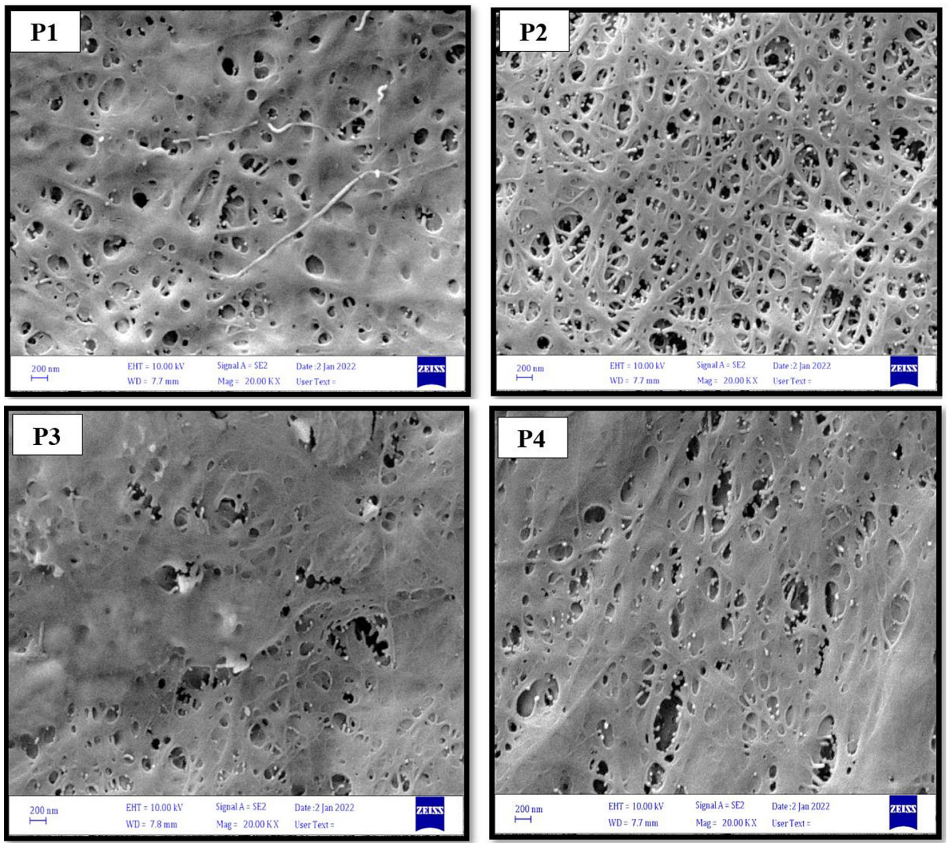

Figure 9. Morphological images of samples from sets P1–P4

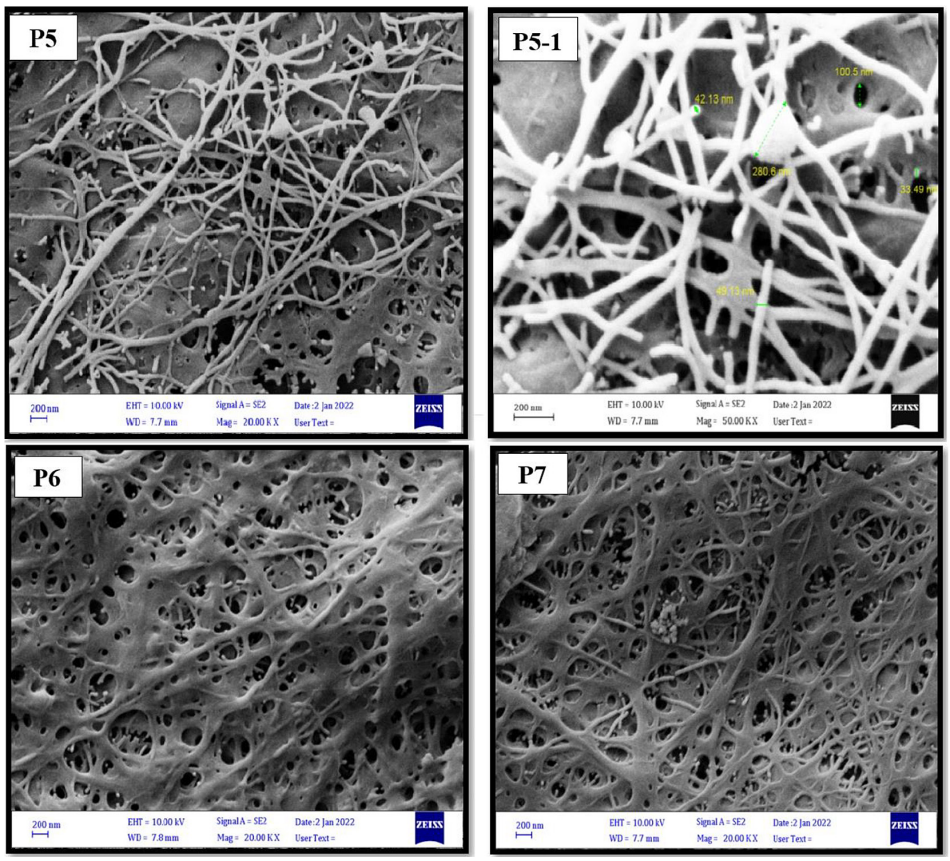

Figure 10. Morphological images of samples from sets P5–P7

control parameters and conditions. Especially, doubling the DP juice ratio in the culture medium for sample P5 increased the porosity and regularity of the porous structure in the produced BNC compared to that produced in sample P3. Sample P3 contains half the DP juice ratio and the same initial pH and incubation temperature. Notably, the pore size is generally 100 nm or smaller. Accordingly, increasing the ratio of DP juice in the samples had a positive effect on the structure and morphology of BNC (Figures 9, 10).

CONCLUSIONS

Based on the findings of this study, the following conclusions can be drawn:

1. Safe disposal of date processing wastes specially DP by reusing it in the substrate for the production of BNC has been proved.
2. Increasing the ratio of DP juice in the substrate has a positive effect on the structure and morphology of the produced BNC.
3. Increasing juice ratio in the medium decreased the yield, water holding capacity and moisture content of the produced BNC
4. The optimal initial pH value and incubation temperature for BNC production were 6 and 30 °C respectively.

REFERENCES

1. Abol-Fotouh, D., Hassan, M.A., Shokry, H., Roig, A., Azab, M.S. and Kashyout, A.E.H.B., 2020. Bacterial nanocellulose from agro-industrial wastes: Low-cost and enhanced production by Komagataeibacter saccharivorans MD1. Scientific Reports, 10(1), 3491.
2. Agarwal, H., Kumar, S.V. and Rajeshkumar, S., 2017. A review on green synthesis of zinc oxide nanoparticles – An eco-friendly approach. Resource-Efficient Technologies, 3(4), 406–413.
3. Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M. and Al-Rawahy, F., 2007. Compositional and functional characteristics of dates, syrups, and their by-products. Food Chemistry, 104(3), 943–947.
4. Al-Hajaya, M., Aljbour, S., Al-Hamaiedeh, H., Abuzaid, M., El-Hasan, T., Hemidat, S. and Nassour, A., 2021. Investigation of energy recovery from municipal solid waste: A case study of al-Karak City Jordan. Civil and Environmental Engineering, 17(2), 610–620.
5. Al-Hamaiedh, H. 2010. Reuse of marble sludge slime in ceramic industry. Jordan Journal of Civil Engineering, 4(3), 264-271.
6. Al-Hamaiedeh, H.D. and Khushefati, W. 2013. Granite sludge reuse in mortar and concrete. Journal of Applied Sciences, 13(3), 444-450.
7. Al-Jrajreh, S.S., Al-Hamaiedeh, H., Al-Kheetan, M.J., Jweihan, Y.S., and Aljaafreh, T. 2023. Improvement of ornamental stone wastes as sand replacement in concrete using silane coupling agent. Results in Engineering, 20, 101580.
8. Al-Awabdeh, F.W., Al-Kheetan, M.J., Jweihan, Y. S., Al-Hamaiedeh, H., and Ghaffar, S.H. 2022. Comprehensive investigation of recycled waste glass in concrete using silane treatment for performance improvement. Results in Engineering, 16, 100790.
9. Aljbour, S.H., Al-Hamaiedeh, H., El-Hasan, T., Hayek, B.O., Abu-Samhadaneh, K., Al-Momany, S., and Aburawaa, A. 2021. Anaerobic co-digestion of domestic sewage sludge with food waste: Incorporating food waste as a co-substrate under semi-continuous operation. Journal of Ecological Engineering, 22(7), 1-10.
10. Alkaabi, J.M., Al-Dabbagh, B., Ahmad, S., Saadi, H.F., Gariballa, S. and Ghazali, M.A., 2011. Glycemic indices of five varieties of dates in healthy and diabetic subjects. Nutrition Journal, 10(1), 1–9.
11. Almeida, I.F., Pereira, T., Silva, N.H.C.S., Gomes, F.P., Silvestre, A.J.D., Freire, C.S.R., Lobo, J.S. and Costa, P.C., 2014. Bacterial cellulose membranes as drug delivery systems: An in vivo skin compatibility study. European Journal of Pharmaceutics and Biopharmaceutics, 86(3), 332–336.
12. Al-Maleki, S., Al-Shamary, E., 2013. Identify some of the properties of bacterial cellulose produced from dates syrup and assessing some of the factors affecting the porosity and thickness of the membranes cellulose. Euphrates Journal of Agriculture Science, 5(3).
13. Al-Nawaeseh, S., Al-Jboor, H., Al-Hamaiedeh, S., Hmeidat, S., and Nassour, A., 2021. Composting of organic waste: A sustainable alternative solution for solid waste management in Jordan. Jordan Journal of Civil Engineering, 15(3).
14. Al-Swalqah, R.A., Al-Kheetan, M.J., Jweihan, Y.S., and Al-Hamaiedeh, H., 2023. Synergistic effect of treated polypropylene-based disposable face masks on durability and mechanical properties of concrete. Arabian Journal for Science and Engineering, 1–9.
15. Aljbour, S.H., Al-Hamaiedeh, H., El-Hasan, T., Hayek, B.O., Abu-Samhadaneh, K., Al-Momany, S., and Aburawaa, A. 2021. Anaerobic co-digestion of domestic sewage sludge with food waste: Incorporating food waste as a co-substrate under semi-continuous operation. Journal of Ecological Engineering, 22(7), 1-10.
16. Alsafadi, D., Ibrahim, M.I., Alamry, K.A., Hussein, M.A. and Mansour, A., 2020. Utilizing the crop

waste of date palm fruit to biosynthesize polyhydroxyalkanoate bioplastics with favorable properties. Science of the Total Environment, 737, 139716.

17. Alva, V.A. and Peyton, B.M., 2003. Phenol and catechol biodegradation by the haloalkaliphile Halomonas campisalis: influence of pH and salinity. Environmental Science & Technology, 37(19), 4397–4402.
18. Bamigbade, G.B., Subhash, A.J., Kamal-Eldin, A., Nyström, L., and Ayyash, M., 2022. An updated review on prebiotics: insights on potentials of food seeds waste as source of potential prebiotics. Molecules, 27(18), 5947.
19. Bæcklund, M., Pedersen, N.L., Björkman, L. and Vahter, M., 1999. Variation in blood concentrations of cadmium and lead in the elderly. Environmental Research, 80(3), 222–230.
20. Barreveld, W.H., 1993. Date palm products. FAO.
21. Carpenter, A.W., de Lannoy, C.F. and Wiesner, M.R., 2015. Cellulose nanomaterials in water treatment technologies. Environmental Science & Technology, 49(9), 5277–5287.
22. Chawla, P.R., Bajaj, I.B., Survase, S.A. and Singhal, R.S., 2009. Fermentative production of microbial cellulose. Food Technology and Biotechnology, 47(2), 107–124.
23. Cheok, C.Y., Mohd Adzahan, N., Abdul Rahman, R., Zainal Abedin, N.H., Hussain, N., Sulaiman, R. and Chong, G.H., 2018. Current trends of tropical fruit waste utilization. Critical Reviews in Food Science and Nutrition, 58(3), 335–361.
24. Czaja, W.K., Young, D.J., Kawecki, M. and Brown, R.M., 2007. The future prospects of microbial cellulose in biomedical applications. Biomacromolecules, 8(1), 1–12.
25. de Azeredo, H.M., 2013. Antimicrobial nanostructures in food packaging. Trends in Food Science & Technology, 30(1), 56–69.
26. Delmer, D.P. and Amor, Y., 1995. Cellulose biosynthesis. The Plant Cell, 7(7), 987.
27. Faostat, 2019. FAOSTAT: Statistical database.
28. Goh, W.N., Rosma, A., Kaur, B., Fazilah, A., Karim, A.A. and Bhat, R., 2012. Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. International Food Research Journal, 19(1).
29. Gullo, M., Sola, A., Zanichelli, G., Montorsi, M., Messori, M. and Giudici, P., 2017. Increased production of bacterial cellulose as starting point for scaled-up applications. Applied Microbiology and Biotechnology, 101, 8115–8127.
30. Hasanzadeh, V., Rahmanian, O. and Heidari, M., 2020. Cefixime adsorption onto activated carbon prepared by dry thermochemical activation of date fruit residues. Microchemical Journal, 152, 104261.
31. Heidarinejad, Z., Rahmanian, O. and Heidari, M., 2019. Production of KOH-activated carbon from date press cake: effect of the activating agent on its properties and Pb (II) adsorption potential. Desalination and Water Treatment, 165, 232–243.
32. Heidarinejad, Z., Rahmanian, O., Fazlzadeh, M. and Heidari, M., 2018. Enhancement of methylene blue adsorption onto activated carbon prepared from Date Press Cake by low frequency ultrasound. Journal of Molecular Liquids, 264, 591–599.
33. Johnson, D.V., 2014. Dates: Postharvest science, processing technology and health benefits. Emirates Journal of Food and Agriculture, 26(11), 1028.
34. Jozala, A.F., Pértile, R.A.N., dos Santos, C.A., de Carvalho Santos-Ebinuma, V., Seckler, M.M., Gama, F.M. and Pessoa, A., 2015. Bacterial cellulose production by *Gluconacetobacter xylinus* by employing alternative culture media. Applied Microbiology and Biotechnology, 99, 1181–1190.
35. Keshk, S.M., Razek, T.M. and Sameshima, K., 2006. Bacterial cellulose production from beet molasses. African Journal of Biotechnology, 5(17).
36. Khleifat, K.M., 2006. Biodegradation of phenol by *Ewingella americana*: Effect of carbon starvation and some growth conditions. Process Biochemistry, 41(9), 2010–2016.
37. Khleifat, K.M., 2007. Biodegradation of phenol by *Actinobacillus sp*.: Mathematical interpretation and effect of some growth conditions. Bioremediation Journal, 11(3), 103–112.
38. Khleifat, K.M., Sharaf, E.F. and Al-limoun, M.O., 2015. Biodegradation of 2-chlorobenzoic acid by Enterobacter cloacae: Growth kinetics and effect of growth conditions. Bioremediation Journal, 19(3), 207–217.
39. Kiziltas, E.E., Kiziltas, A. and Gardner, D.J., 2015. Synthesis of bacterial cellulose using hot water extracted wood sugars. Carbohydrate Polymers, 124, 131–138.
40. Léonard, D. and Lindley, N.D., 1999. Growth of Ralstonia eutropha on inhibitory concentrations of phenol: diminished growth can be attributed to hydrophobic perturbation of phenol hydroxylase activity. Enzyme and Microbial Technology, 25(3–5), 271–277.
41. Loh, K.C. and Wang, S.J., 1997. Enhancement of biodegradation of phenol and a nongrowth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. Biodegradation, 8, 329–338.
42. Majzoobi, M., Karambakhsh, G., Golmakani, M.T., Mesbahi, G. and Farahnaky, A., 2020. Effects of level and particle size of date fruit press cake on batter rheological properties and physical and nutritional properties of cake. Journal of Agricultural Science and Technology, 22(1), 121–133.
43. Majzoobi, M., Karambakhsh, G., Golmakani, M.T., Mesbahi, G.R. and Farahnaki, A., 2019. Chemical composition and functional properties of date press

cake, an agro-industrial waste. Journal of Agricultural Science and Technology, 21(7), 1807–1817.

44. Mazaheri, A.M. and Nikkhah, M., 2002. Production of citric acid from date pulp by solid state fermentation. Journal of Agricultural Science and Technology, 4(3-4), 119-125.
45. Medic, J., 2011. Starch properties, endogenous amylase activity, and ethanol production of corn kernels with different planting dates and drying conditions. Iowa State University.
46. Norouzi, S., Heidari, M., Alipour, V., Rahmanian, O., Fazlzadeh, M., Mohammadi-Moghadam, F., Nourmoradi, H., Goudarzi, B. and Dindarloo, K., 2018. Preparation, characterization and Cr (VI) adsorption evaluation of NaOH-activated carbon produced from Date Press Cake; an agro-industrial waste. Bioresource Technology, 258, 48–56.
47. Oguro, M., Imahiro, S., Saito, S. and Nakashizuka, T., 2021. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1.
48. Plazzotta, S., Manzocco, L. and Nicoli, M.C., 2017. Fruit and vegetable waste management and the challenge of fresh-cut salad. Trends in Food Science & Technology, 63, 51–59.
49. Rambabu, K., Bharath, G., Hai, A., Banat, F., Hasan, S.W., Taher, H. and Mohd Zaid, H.F., 2020. Nutritional quality and physico-chemical characteristics of selected date fruit varieties of the United Arab Emirates. Processes, 8(3), 256.
50. Schramm, M. and Hestrin, S., 1954. Factors affecting production of cellulose at the air/liquid interface of a culture of Acetobacter xylinum. Microbiology, 11(1), 123–129.
51. Son, H.J., Heo, M.S., Kim, Y.G. and Lee, S.J., 2001. Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated Acetobacter. Biotechnology and Applied Biochemistry, 33(1), 1–5.
52. Volova, T.G., Prudnikova, S.V., Sukovatyi, A.G. and Shishatskaya, E.I., 2018. Production and properties of bacterial cellulose by the strain Komagataeibacter xylinus B-12068. Applied Microbiology and Biotechnology, 102, 7417–7428.