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Comparative Effect of Agrowastes on Bacterial Cellulose Production by *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1

B.C. ADEBAYO-TAYO*, M.O. AKINTUNDE, S.O. ALAO

Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria

*Corresponding author: bukola.tayo@gmail.com

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Abstract

Comparative effect of Pineapple waste medium (PIWAM) and Pawpaw waste medium (PAWAM) on the production of biocellulose (BC) by *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 was investigated. The dry weight of the BC produced by *Acinetobacter* sp. BAN1 ranged from 0.4 – 0.6 g l^{-1} and 0.2 – 1.1 g l^{-1} in PIWAM and PAWAM. The dry weight of the BC produced by *Acetobacter pasteurianus* PW1 ranged from 0.1 – 3.9 g l^{-1} and 0.2 – 1.0 g l^{-1} in PIWAM and PAWAM. PIWAM supported the highest BC production by the two strains. 37°C, 35°C and 28°C supported the highest BC production in PIWAM and PAWAM by the isolates. pH 8, pH 3 and pH 7 was the best for BC by *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 in PIWAM and PAWAM. FTIR spectrometry analysis of the BC showed the presence of β- glycosidic bonds connecting the carbohydrate monomers, hydroxyl groups, carbonyl groups and vibrating sugar rings. In conclusion, the study has demonstrated the ability of utilizing low cost agro wastes as substrates for bacterial cellulose production.

Key words: Bacterial cellulose, *Acinetobacter* sp. BAN1, *Acetobacter pasteurianus* PW1, Fruit wastes, FTIR

Introduction

Z Cellulose is a homopolymer consisting of glucose glycosidically linked in a β-1→4 conformation. Cellulose is the most abundant natural biopolymer on earth (Keshk, 2014). Bacterial cellulose (BC) is cellulose synthesized by different microorganisms. BC came as a substitute for plant cellulose to reduced the demand of cellulose from plants (Brown, 2004). BC is a biopolymer produced by several strains of bacteria. Bacteria of the genus *Acetobacter, Achromobacter, Pseudomonas, Azotobacter, Sarcina, Agrobacterium* have the ability to produce BC. Bacteria of the genus *Acetobacter* has been majorly employed in the production of BC because they have superior production ability than other BC-producers (Jung et al., 2005). One of the most important features of BC is its chemical purity, which distinguishes it from plant cellulose that is usually associated with hemicellulose and lignin with difficulty in their removal (Ruka et al., 2012). BC also has high water-binding capacity, durability, elasticity, good shape retention, high crystallinity thn plant cellulose (Bielecki et al., 2005). BC has practical application in biotechnology, biomedicals (George et al., 2005), in making artificial nails, water ultrafiltration, paper production, sport cloths (Bielecki et al., 2005), artificial skin for wound dressing (Fontana et al., 1990) and bone tissue engineering and bone grafting (Yoshikawa and Myoui, 2005), BC-based bloos vessels (Klemm et al., 2001).

The main problem associated with the production of BC is the cost of media. The use of monosaccharides and disaccharides as substrates for production of BC is expensive. The cost of media contributes greatly to the fermentation cost. Research on producing BC utilizing low-cost substrates is to identify how to reduce BC production cost to reduce the cost of BC production, low-cost ready to use substrates are needed. Some cheap agricultural products and wastes can be used as alternative low-cost substrates for BC production since they contain sugars that microorganisms can bioutilize for the production of BC (Bae and Shoda, 2004).

In light of this information, new economical culture media for industrial scale production of BC, studies have focused on agricultural wastes and industrial by-products as potential medium

(Kurosumi et al., 2009; Gomes et al., 2013; Cakar et al., 2014). These agricultural wastes are rich in sugars which can be easily assimilated by microorganisms; this makes them suitable for the industrial production of bioproducts by microorganisms (Rosales et al., 2005). Therefore, rather than the use of pure sugars which are expensive, Agricultural wastes such as fruit wastes (wastes from pulp, skin and discarded fruits) can be used for the production of BC. The use of wastes as substrates reduces environmental pollution and encourages production of BC with less expensive substrate.

This research aimed to investigate the comparative effect of agrowastes on BC production using *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1.

Materials and Methods

Sample collection*:* Pineapple and papaw (peel and pulp) were gotten from the prepared fruit juice. The substrate were oven dried at 60°C for 24 hrs, blended and stored in a dry air-tight containers for further studies.

Culture maintenance: Stock culture of microorganisms (*Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1) obtained from the Department of Microbiology, University of Ibadan were maintained on slants of Hestrin-Schramm (HS) medium composed of glucose (2g), yeast extract (0.5g), peptone (0.5g), citric acid (0.12g), disodium hydrogen phosphate (0.27g), Agar (1.0g). The isolates were subcultured onto fresh HS- agar before use (Hestrin and Schramm, 1954).

Production of bacterial cellulose using agro waste: Production of bacterial cellulose using agrowastes was done according to the method of (Kamarudin et al., 2013). Seed broth was prepared by inoculating the isolates into 10 ml tubes containing HS broth and were incubated at 30°C for 3-5 days. 5 ml^{-1} of the seed culture was inoculated into the Production Medium containing; Na_2HPO_4 (0.34 g), peptone (0.62 g), yeast extract (0.62 g), citric acid (0.14 g), dried fruit wastes (2.0 g) and distilled water was added to make upto 50 mls at pH 5.0. The inoculated production medium was incubated statically at 28-30°C for 15 days. The pH, growth and the biocellulose produced was characterized.

Effect of incubation time on the production of bacterial cellulose: The effect of incubation time on BC production was determined by incubating the inoculated production media at different time interval of 5-15 days. The growth of the organisms, pH of the fermenting medium and the biocellulose produced were monitored over a period of 15 days, checking at interval of 5 days. Biocellulose produced was characterized by determining the Reducing sugar, dry weight, FTIR and SEM spectroscopy.

Effect of temperature on production of bacterial cellulose: The effect of temperature on BC production was determined by incubating the inoculated production media at different temperature (28°C, 35°C, 37°C) for 15 days. The biocellulose produced was characterized by determining the Reducing sugar, dry weight, FTIR and SEM spectroscopy.

Effect of pH on the production of bacterial cellulose: The effect of pH on BC production was done by adjusting the pH range (pH 3, 5, 7 and 8) of the production media. The pH adjusted production media was sterilized, inoculated and incubated at 30°C for 15 days. The biocellulose produced was characterized by determining the Reducing sugar, dry weight, FTIR and SEM spectroscopy.

Characterization of the BC

Reducing sugar analysis: The BC produced was quantified by determining the reducing sugar in the fermentation broth (Miller, 1959).

Dry weight measurement: Dry weight of the pellicles produced after fermentation was determined according to the method of (Aydin and Aksoy 2009). The produced cellulose was washed repeatedly with distilled water and dried at 70°C to a constant weight and measured using weighing balance.

Fourier transformed infra-red spectroscopy (FTIR) of the biocellulose: The cellulose samples obtained from the fermentation medium was analysed to study conformational characteristics by FTIR spectrometer using KBr plate method (Gayathry and Gopalaswamy 2014).

Scanning electron microscope images of BC: The purified BC pellicles was viewed using a scanning electron microscope (ASPEX 3020) to observe the formation and type of cellulose fibres produced by the isolates. The morphological characterization of the cellulose fibrils was done using scanning electron microscope (SEM) (Gayathry and Gopalaswamy, 2014).

Result and Discussion

The comparative effect of agrowastes (Pineapple waste medium (PIWAM) and Pawpaw waste medium (PAWAM) on the growth and BC production at different time interval was investigated. Figure 1 shows the comparative effect of the Agrowaste on the growth of *A.* sp. BAN1 and *A. pastuerianus* PW1. In PIWAM and PAWAM, the growth of *A.* sp. BAN1 ranged from 0.478 – 1.668 and 0.538 – 1.931. The highest was recorded at day 5 of incubation. At day 0, 5 and 15 of fermentation, PAWAM supported the highest growth, while at day 10 of fermentation, PIWAM supported the highest growth. The growth of *A. pastuerianus* PW1 in PIWAM and PAWAM ranged from 0.430 – 1.232 and 0.366 – 1.430 the highest was recorded at day 5 of incubation. At day 0 and 5, PAWAM supported the highest growth, while at day 10 and 15, PIWAM supported the highest growth.

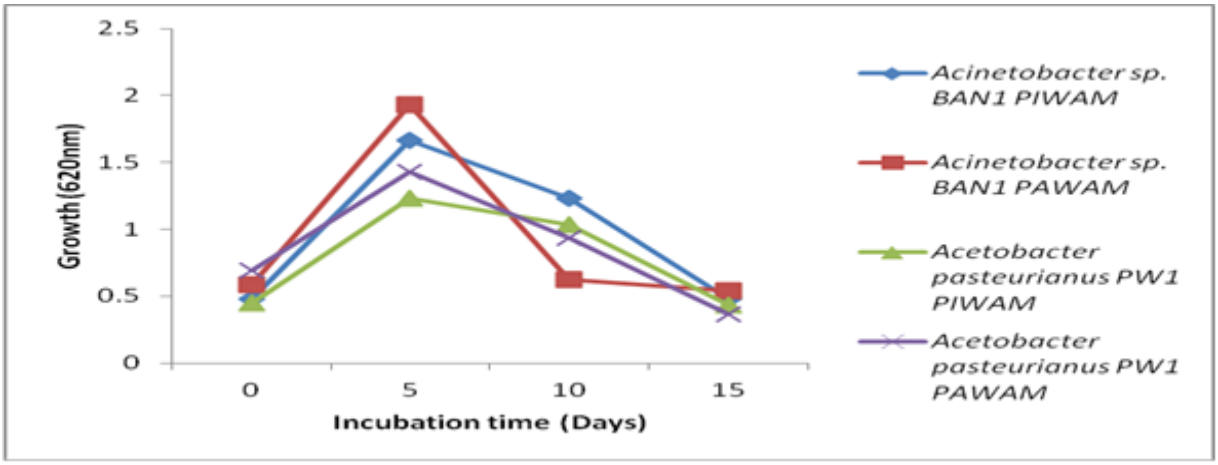

Figure1. Growth of *A.* sp. BAN1 and *Acetobacter pasteurianus* PW1 in PIWAM and PAWAM at different incubation time (days)

The selected agrowastes used for this research work supported the growth of the BC producing strains. The isolates had the ability to utilize the agrowastes for both cell metabolism and BC formation. The ability of PAWAM and PIWAM to support the high production of BC may be as a result of their chemical composition which include sugars and minerals which may enhance BC production.

The activities of the cells in the medium varied during the period of fermentation and the sugars in the medium were reduced. The cell growth negatively correlates with the reducing sugars. The reduction in the sugar shows the activity of microorganisms in the media. Even though the maximum growth was at day 5, the microorganisms still actively reduced the sugars in the medium. The reduction in sugars relates to substrate utilization by the microorganisms and also formation of bacterial cellulose. This means that the formation of BC may be predicted based on the pattern of substrate utilization. This study showed that maximum BC yield was achieved at day 10, which is in accordance with the work of Lestari et al., (2014) that achieved a maximum BC yield at day 12, and maximum cell growth at day 6.

Figure 2 shows pH of the fermenting medium, using PIWAM and PAWAM for the production of BC at different incubation time (Days). During fermentation at different time intervals in PIWAM and PAWAM inoculated with *A.* sp. BAN1. The pH ranged from 4.6 – 5.3 and 4.3 – 5.2. The lowest pH was recorded in PAWAM. The highest pH was recorded in PIWAM. Generally, at day 0 – 15, there was significant difference ($P \leq 0.05$) in the pH development during the fermentation using PIWAM and PAWAM. There was no significant difference in the pH development during fermentation using PAWAM at day 10 – 15.

During fermentation at different time intervals in PIWAM and PAWAM inoculated with *A. pasteurianus* PW1, the pH ranged from 4.9 – 7.2 and 4.6 – 6.2. The lowest pH was recorded in PAWAM. The highest pH was recorded in PIWAM. Generally, at day 0 – 15, there was significant difference ($P \leq 0.05$) in the pH development during the fermentation using PIWAM and PAWAM.

A significant increase in pH was recorded in the PIWAM and PAWAM inoculated with A. *pasteurianus* PW1 after the 5th day of incubation, which could be due to the activities of the isolate and adaptation to the environment. The pH increased from slightly acidic to neutral pH. This condition may favour the formation of bacterial cellulose better than an acidic condition, because a neutral pH provides a mild and flexible

environment or chance for the microorganisms to adapt and promote growth and bioconversion of polysaccharide (Kamarudin et al., 2013). The pH of PIWAM and PAWAM inoculated with *A.* sp. BAN1 maintained an acidic pH all through the fermentation process, which may have been due to the activities of the microorganism. The pH was in the range of the optimum pH (pH 3.5 – 6.0) for the production of BC (Pae et al., 2011).

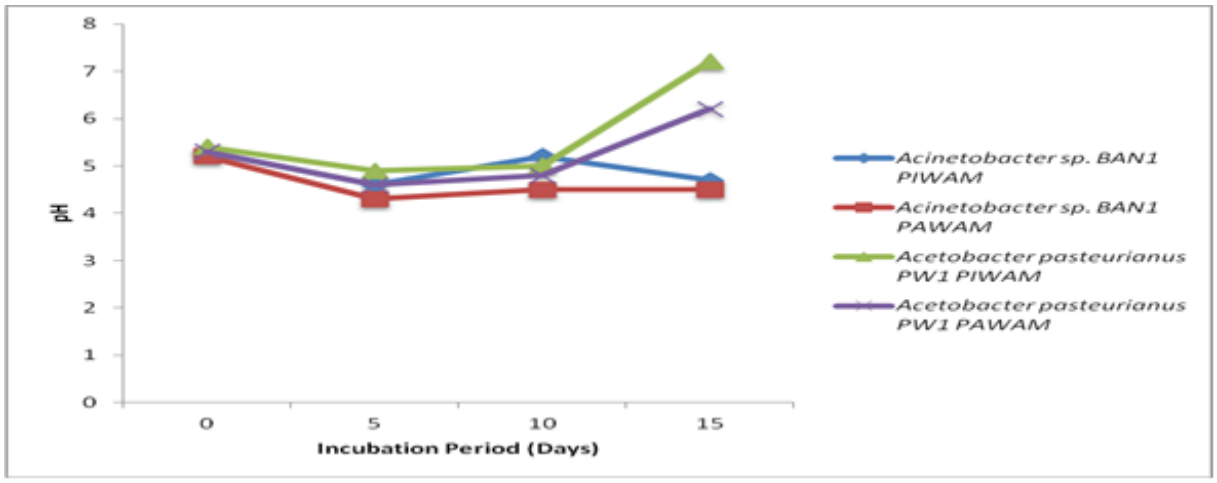

Figure 2. pH of fermenting media using wastes as substrate inoculated with *A.* sp. BAN1 and *A. pasteurianus* PW1 during the period of production of bacterial cellulose

Table 1 shows the Bacterial cellulose yield (mg l^{-1}) at different incubation days during the production by BAN1 and *A. pasteurianus* PW1 using PIWAM and PAWAM. For *A.* sp. BAN1, in PIWAM and PAWAM, the BC yield ranged from 2.77 – 8.10 mg $l^{-1}$and 2.36 – 4.74 mg l^{-1}. The highest was recorded at day 5 and the lowest was recorded at day 15. At day 0 – 15 of fermentation, PIWAM supported the highest BC yield. For *A. pasteurianus* PW1, in PIWAM and PAWAM, the BC yield ranged from 3.67 – 8.13 mg l^{-1} and 2.88 – 8.63 mg l^{-1}. The highest was recorded at day 5 and the lowest at day 15. At day 5 of fermentation, PAWAM supported the highest BC yield, while at day 10 – 15 of fermentation, PIWAM supported the highest yield.

Table 1. Bacterial cellulose yield (mg l^{-1}) during the period of fermentation, using agrowastes by *A.* sp. BAN1 and *A. pasteurianus* PW1

Incubation time (days)	*A.* sp. BAN 1 Reducing sugar (mg l^{-1})		*A. pasteurianus* PW 1 Reducing sugar (mg l^{-1})	
	PIWAM	PAWAM	PIWAM	PAWAM
0	0.00	0.00	0.00	0.00
5	8.10	4.74	8.13	8.63
10	4.50	3.08	5.72	5.44
15	2.77	2.36	3.67	2.88

Figure 3 shows the dry weight of BC produced by *A.* sp. BAN1 and *A. pasteurianus* PW1 using PIWAM and PAWAM. In PIWAM, the dry weight of BC produced by *A.* sp. BAN1 ranged from 0.4 – 0.6 g l^{-1}. The highest BC was recorded at day 10 and the least BC was recorded at day 5 and 15. In PAWAM, the dry weight of BC produced ranged from 0.2 – 1.1 g l^{-1}. The highest BC was recorded at day 10. The lowest BC was recorded at day 15. At day 5 and 10, PAWAM supported the highest BC production, while at day 15, PIWAM supported the highest BC production.

In PIWAM and PAWAM, the dry weight of BC produced by *A. pasteurianus* PW1 ranged from 0.1 – 3.9 g l^{-1} and 0.2 – 1.0 g l^{-1}. The highest BC was recorded at day 10 and day 5 respectively and the lowest BC was recorded at day 15. At day 5 and 10, PIWAM supported the highest BC production while at day 15, PAWAM supported the highest BC production.

Figure 4 shows the FTIR spectra of BC produced using agrowastes. The FTIR spectra of BC produced by *A.* sp. BAN1 in PIWAM and PAWAM is shown in Figure 4 (a - b). The distinguishing peak at 3441.12 cm^{-1} in PIWAM and at 3383.26 cm^{-1} in PAWAM indicates O – H stretching. Peak at 2852.81 cm^{-1} – 2924.18 cm^{-1} in PIWAM and at 2854.74 cm^{-1} – 2924.18 cm^{-1} in PAWAM indicates C

– H stretching. Peak at 1653.05 cm^{-1} in PIWAM and at 1635.69 cm^{-1} in PAWAM indicates presence of carbonyl group (C = O). Peak at 1039.67 cm^{-1} – 1058.96 cm^{-1} in PIWAM and at 1035.81 cm^{-1} – 1058.96 cm^{-1} in PAWAM indicates C – O stretching. Peak at 1406.15 cm^{-1} in PIWAM and at 1415.8 cm^{-1} in PAWAM indicates CH_2 bending. Peak at 1319.35 cm^{-1} in PIWAM and at 1319.35 cm^{-1} in PAWAM indicates C – H bending. Peak at 1159.26 cm^{-1} in PIWAM and at 1157.33 cm^{-1} in PAWAM indicates C – O – C stretching.

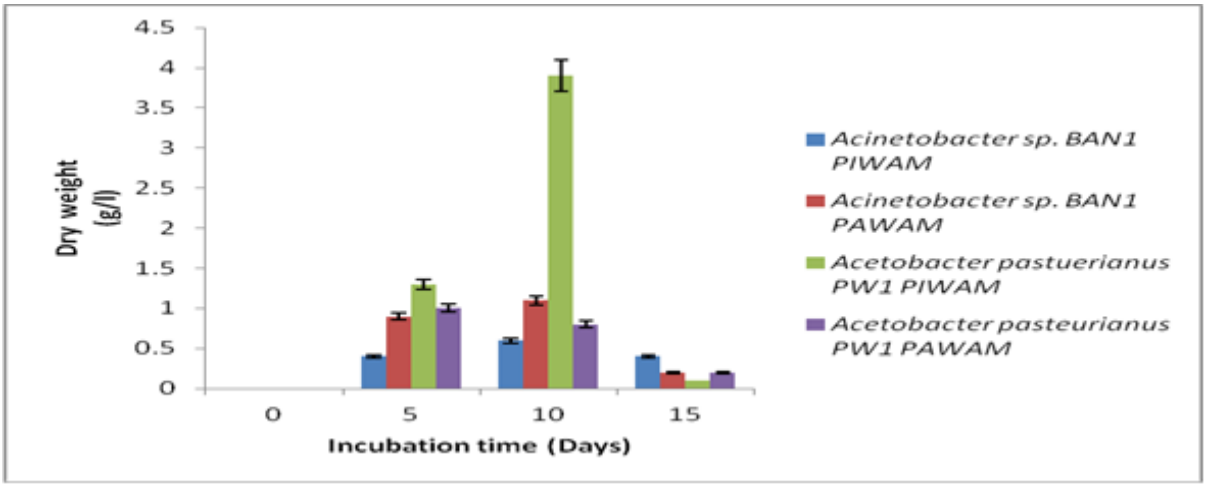

Figure 3. Dry weight of Bacterial cellulose produced at different incubation time in the media using wastes as substrate by *A.* sp. BAN1 and *A. pasteurianus* PW1

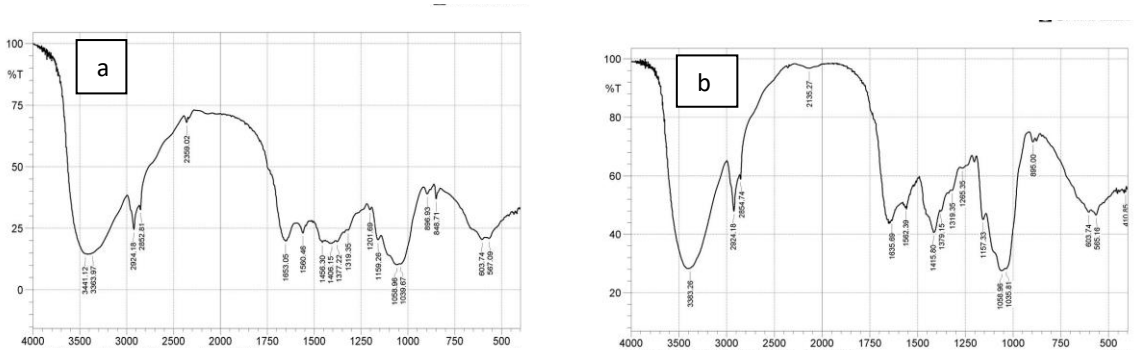

Figure 4. FTIR spectra of bacterial cellulose produced by *A.* sp. BAN1 using (a) PIWAM and (b) PAWAM

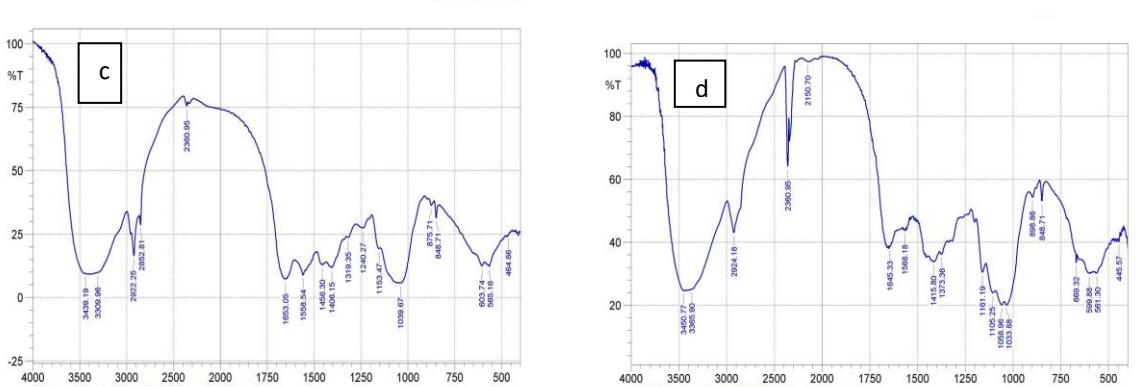

Figure 4. FTIR spectrum of bacterial cellulose produced by *A. pasteurianus* PW1 using (c) PIWAM and (d) PAWAM

The position and intensity of absorption bands of a substance are specific. Infra-red spectroscopy by FTIR is highly characteristic for a substance (Gunzler and Gremlich, 2002). FTIR analyzes cellulose using the chemical bonding that present in the polymer even though the peaks and curves may change, either increase or decrease in wavewlenght when the cellulose structure is changing due to the substrate used for production or treatment applied (Oh et al., 2005; Sun et al.,

2008). There are important bonds in cellulose polymer that confirms the production of cellulose. According to previous BC research, spectrum with peaks at 1160 cm^{-1} and 900 cm^{-1} which indicates the bonding in cellulose where the carbohydrate monomers connect into a polymer with the C-O-C bonding notation (Sun et al., 2008). Also, strong peak that appear at 1060 cm^{-1} and 1030 cm^{-1} are the indicative of C-O stretching. The absorption peak of carbonyl groups (C=O) with intramolecular hydrogen bonds is also found at around 1650 cm-1 (Guo and Wu, 2008). Distinguish peaks of 3350 cm^{-1} and shouldering around 3400 cm^{-1} to 3500 cm^{-1} can be associated to the hydroxyl bonds. The spectra region can be referred to as the intermolecular and intermolecular hydrogen bonds of cellulose.

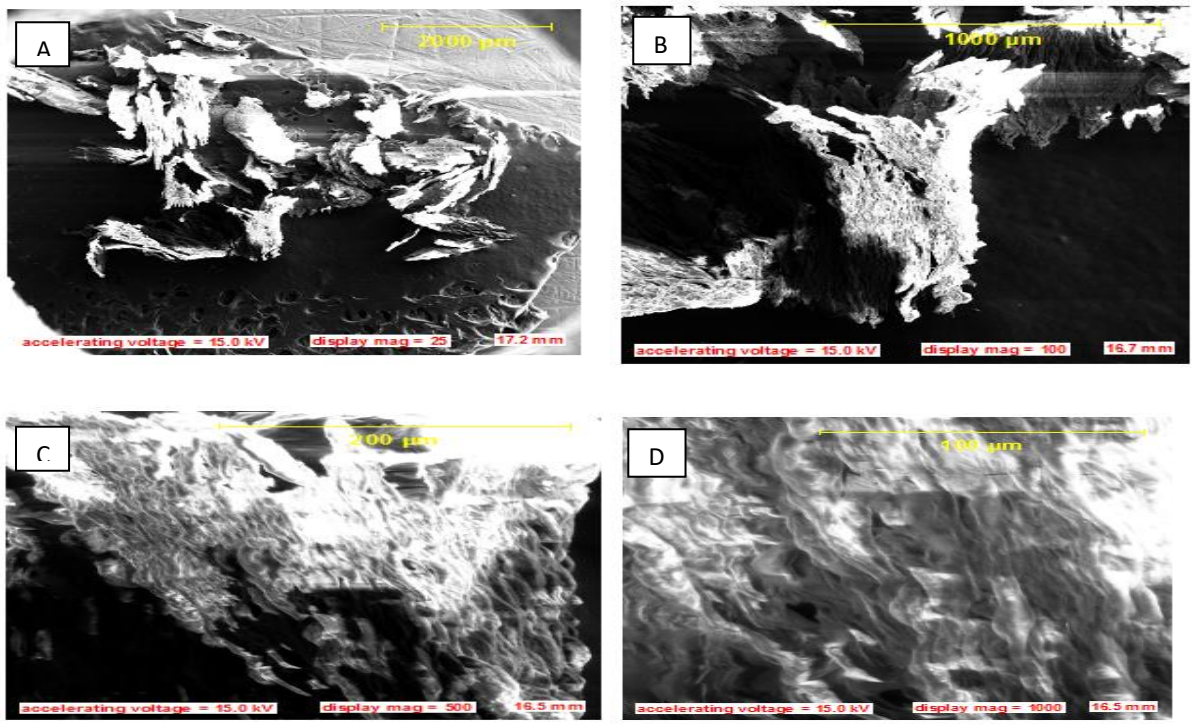

Figure 7. SEM image of Bacterial cellulose produced by *Acetobcter pasteurianus* PW1 using PIWAM at magnifications; (A) 25X, (B) 100X, (C) 500X and (D) 1000X

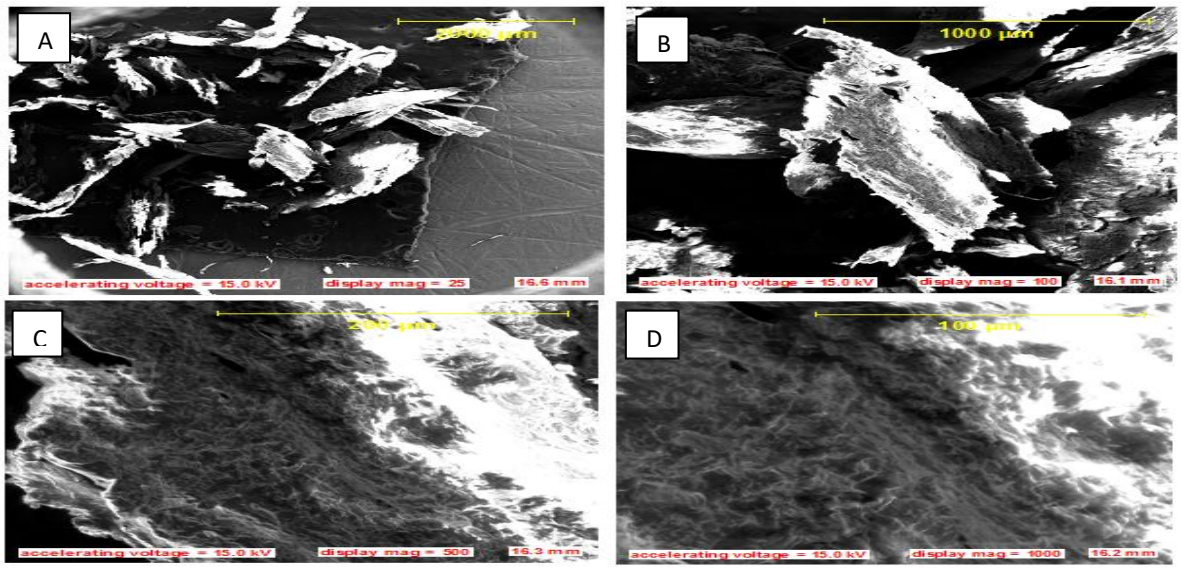

Figure 8. SEM image of bacterial cellulose produced by *A.* sp. BAN1 using PAWAM at magnifications; (A) 25X, (B) 100X, (C) 500X and (D) 1000X

According to Marchessault and Sundararajan, (1983), pure cellulose spectrum had distinguish peaks of 3350 cm^{-1} and shouldering around 3400 cm^{-1} to 3500 cm^{-1} indicates O-H stretching, 2800 cm^{-1} 1 to 2900 cm^{-1} indicates C-H stretching, 1160 cm^{-1} indicates C-O-C stretching and 1035 cm^{-1} to 1060 cm^{-1} indicates C-O stretching. Other fingerprint regions for cellulose are peaks around 1300 cm^{-1} indicating C-H bending and around 1400 cm^{-1} indicating CH_2 bending. BC produced from *A.* sp. BAN1 and *A. pasteurianus* PW1 in PIWAM and PAWAM, showed peaks that correspond with that of pure cellulose. This confirms that there is a similarity between the cellulose produced by the isolates in this study and pure cellulose. The SEM analysis showed that the BC surface was unsmooth and layered. Klemm et al. (2001) reported that biocellulose is a layered formation.

Table 2 shows the comparative effect of temperature on BC yield (mg l^{-1}) by *A.* sp. BAN1 and *A. pasteurianus* PW1 using agrowastes (PIWAM and PAWAM) as substrate in submerged fermentation. For *A.* sp. BAN1. In PIWAM and PAWAM, BC yield ranged from 4.80 – 4.97 mg $l^{-1}$and 4.90 – 6.54 mg l^{-1}. The highest yield was supported by 37°C. The lowest yield was recorded at 28°C and 35°C respectively. At the different temperature of incubation, PAWAM supported the best BC yield.For *A. pasteurianus* PW1, BC yield ranged from 3.78 – 4.34 mg l^{-1} and 4.78 – 6.46 mg l^{-1}. The highest yield was recorded at 37°C in PIWAM and 35°C in PAWAM. The lowest yield was recorded at 28°C and 37°C.

Table 2. Comparative effect of temperature on bacterial cellulose yield (mg l^{-1}) by *A.* sp. BAN1 and *A. pasteurianus* PW1 using Agrowastes as substrate

Temperature ranges	BC yield (mg l^{-1})		Reducing sugar	
	PIWAM	PAWAM	PIWAM	PAWAM
28°C	4.80	5.78	3.78	5.02
35°C	4.85	4.90	4.23	6.46
37°C	4.97	6.54	4.34	4.78

KEY: PIWAM- Pineapple Waste Medium, PAWAM- Pawpaw Waste Medium

Figure 5 shows the comparative effect of temperature on the dry weight of BC produced, using agrowastes (PIWAM and PAWAM) by *A.* sp. BAN1 and *A. pasteurianus* PW1. The dry weight of BC produced by *A.* sp. BAN1 in PIWAM ranged from 0.3 – 1.0g l^{-1}. The highest was recorded at 37°C, followed by 28°C (0.5g l^{-1}) and 35°C recorded the lowest weight. In PAWAM, the dry weight of BC ranged from 0.2 – 0.8g l^{-1}. The highest BC was recorded at 37°C and 28°C and 35°C recorded the lowest BC. At all the different temperature ranges, PIWAM supported the highest dry weight. The dry weight of BC produced by *A. pasteurianus* PW1 in PIWAM, ranged from 0.1 – 0.5g l^{-1}. The highest BC was recorded at 28°C, followed by 35°C (0.2g l^{-1}) and 37°C recorded the lowest BC. In PAWAM, the dry weight of BC ranged from 0.2 – 0.7 g l^{-1}, the highest BC was recorded at 35°C and the lowest BC weight was recorded at 28°C and 37°C. At 28°C, PIWAM supported the highest dry weight. At 35°C and 37°C PAWAM supported the highest BC production.

Table 3 shows the comparative effect of pH on the Bacterial cellulose yield (mg l^{-1}), by *A.* sp. BAN1 and *A. pasteurianus* PW1, using PIWAM and PAWAM. For *A.* sp. BAN1. In PIWAM and PAWAM, the BC yield ranged from 3.34 – 5.49 mg l^{-1} and 4.24 – 6.75 mg l^{-1}. The highest was recorded in pH 7 and pH 8 respectively and the lowest was recorded at pH 5. At pH 3, 5 and 8, PAWAM supported the best BC yield while at pH 7, PIWAM supported the best BC yield. For *A. pasteurianus* PW1. In PIWAM and PAWAM, the BC yield ranged from 2.72 – 5.25 mg $l^{-1}$and 3.45 – 7.24 mg l^{-1}. The highest yield was recorded at pH 8 and pH 7 respectively. The lowest yield was recorded at pH3. At the different pH ranges, PAWAM supported the best BC yield.

Figure 6 shows the comparative effect of pH on the dry weight of BC produced by *A.* sp. BAN1 and *A. pasteurianus* PW1, using PIWAM and PAWAM. The dry weight of BC produced by *A.* sp. BAN1 in PIWAM and PAWAM ranged from 0.1 – 2.0 g $l^{-1}$and 0.2 – 2.1 g l^{-1}. The highest BC weight was recorded at pH 8 and pH 3 respectively. The lowest weight was recorded at pH 5. At pH 3, 5 and 7, PAWAM supported the highest BC weight while at pH 8, PIWAM supported the highest weight. The dry weight of BC produced by *A. pasteurianus* PW1 in PIWAM and PAWAM ranged from 0.1 – 1.0 g l^{-1} and 0.1 – 0.6 g l^{-1}. The highest weight was recorded at pH 8 and pH 7 respectively. The lowest BC weight was recorded at pH 3. At pH5 and 7, PAWAM supported the highest BC weight. At pH8, PIWAM supported the highest BC weight.

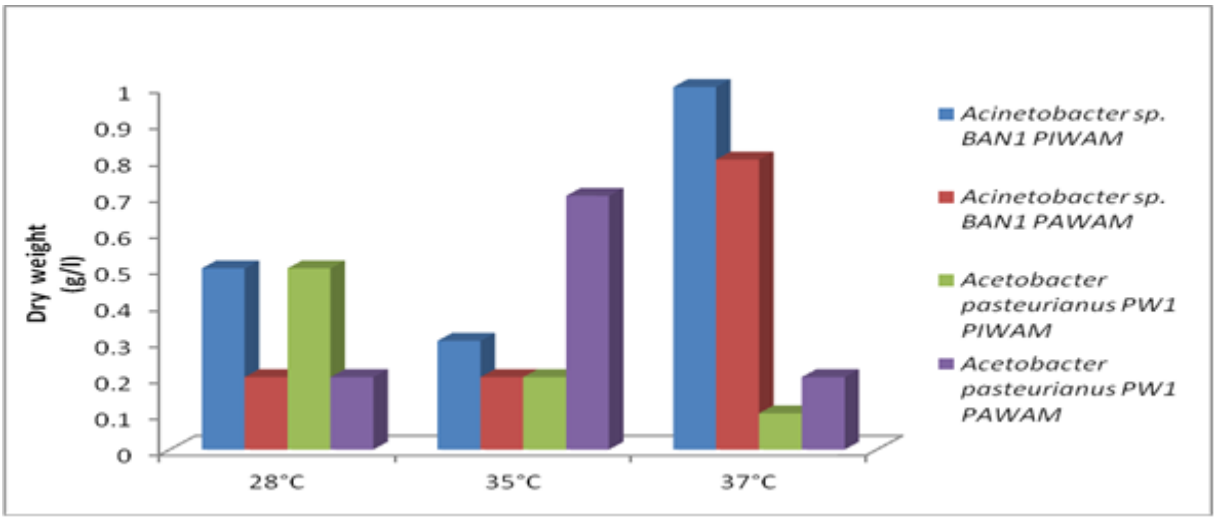

Figure 5. Comparative effect of Temperature on BC produced using agrowaste as substrate by *A. pasteurianus* PW1 and *A.* sp. BAN1

Table 3. Comparative effect of pH on Bacterial cellulose yield (mg l^{-1}) by *A.* sp. BAN1 and *A. pasteurianus* PW1 using Agrowaste as substrate

pH	BC yield (mg l^{-1})			
	Acinetobacter sp. BAN 1		*Acetobacter pasteurianus* PW 1	
	PIWAM	PAWAM	PIWAM	PAWAM
pH 3	3.54	4.32	2.72	3.45
pH 5	3.34	4.24	3.85	4.66
pH 7	5.49	4.93	4.27	7.24
pH 8	5.03	6.75	5.25	5.72

KEY: PIWAM- Pineapple Waste Medium, PAWAM- Pawpaw Waste Medium

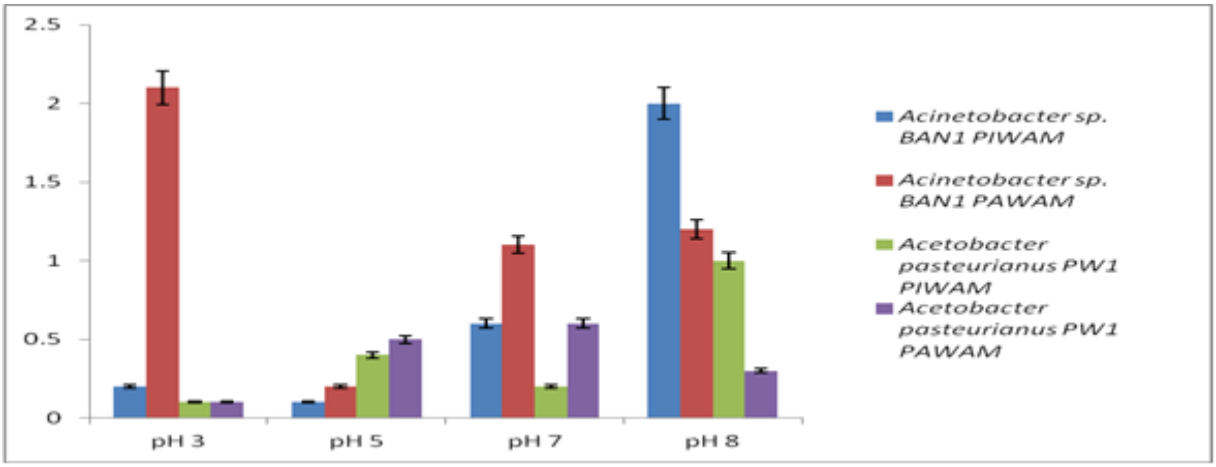

Figure 6. Comparative effect of pH on dry weight of Bacterial cellulose produced by *A.* sp. BAN1 and *A. pasteurianus* PW1 using Agrowastes

The ability of 37°C to support the best BC production by *A.* sp. BAN1 in both PIWAM and PAWAM is in contrast to the optimal temperature range stated by Jonas and Farah (1998) which was 25-30°C. While the ability of 35°C and 28°C to support the best BC production by *A. pastuerianus* PW1 in PAWAM and PIWAM is in line with the report of (Jonas and Farah 1998).

The ability of pH 8 to support the best production of BC may be as a result of minimal or no activity for conversion of glucose to gluconic acid. This agree with the report of Pourramezan et al., (2009) who reported that alkaline pH is favourable for cellulose production because of the minimum conversion of glucose to gluconic acid which increases cellulose production. The best pH for production of BC by *A. pasteurianus* PW1 in PAWAM was at pH 7, which also agrees with Pourramezan et al., (2009) who after optimization

achieved the best BC yield at pH 7 using *A.* sp. 4B-2 in HS medium.

In conclusion*, Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 are good BC producers. PIWAM was the best substrate for BC production by the two strains. 37°C, 35°C and 28°C. pH 8, pH 3 and pH 7 was the best for BC production by the strains. Functional groups indicating BC was present and the BC was crystalline.

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