Egypt. J. Biotechnol. Vol. 52, June, 2016.

ENHANCEMENT OF BIOCELLULOSE PRODUCTIVITY THROUGH OPTIMIZATION OF CULTURAL CONDITIONS AND GAMMA RADIATION

BY

Mohamed M. Roushdy, Abbas A. El-Ghamery, Ali A. Hammad*, Salwa A. Abou EL-Nour* and Nasser H. Mohammad*

FROM

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

*Microbiology Department, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Cairo, Egypt

ABSTRACT

*The optimum fermentation conditions for the production of biocellulose (BC) by the isolated strain **Komagataeibacter rhaeticus K** were determined. The isolate gave the highest cellulose production when grown on glucose ethanol medium (GEM) and was able to produce cellulose at 20–35°C with a maximum at 30°C. BC production was obtained at pH 3–8 with a maximum at pH 5. Effect of gamma radiation induced by coblet-60 on **K. rhaeticus K** cells was investigated. A cellular survival curve versus absorbed doses was studied to determine the sensitivity of bacterial cells to gamma ray. The results showed D_{10}-value was equals to 0.38kGy. To enhance the ability of **K. rhaeticus K** for BC production, the isolate was subjected to different doses of Gamma ray and the optimum BC yield was obtained at 0.4kGy. The maximum production of BC was obtained with using the improved medium (5% (w/v) glucose, 0.3% (w/v) yeast extract, 0.3% (w/v) peptone and 2% (v/v) ethanol). Using fresh culture of **K. rhaeticus K** that exposed to 0.4kGy of gamma radiation and grown under the optimized culture conditions, 9.3g/l dry cellulose was produced after 7 days of static cultivation, although this isolate produced only 4g/l in the standard medium.*

INTRODUCTION

Cellulose is one of the most important polysaccharide substance common in all plant material. It is a polymer of β-D-glucose units linked together by (1→4) glycosidic bonds to form cellobiose residues that are the repeating units in the cellulose chain (Moon *et al.,* 2011).

Biocellulose or bacterial cellulose (BC) is chemically equivalent to plant cellulose but it has distinct ultra-fine fibrils of nano-sized three-dimension network structure. It possess superior and unique properties compared to plant cellulose such as good mechanical strength, high water absorption capacity (over100 times of its weight), high degree of crystalinity, high polymerization degree, ultra-fine and highly pure network structure, great elasticity, non-drying state and biocompatibility (Keshk and

Sameshima, 2006; George and Siddaramaiah, 2012; Almeida *et al*., 2014). These unique properties have attracted much attention to the use of BC in different applications such as food preparations (Shi *et al.,* 2014), functional papers sheet (Basta and El-Saied, 2009), artificial blood vessels and wound dressing (Keshk, 2014).

BC is an extracellular cellulose produced by some acetic acid bacteria in the genus *Komagataeibacter* (formerly *Gluconacetobacter*) (Yamada*et al*., 2012), such as *K. xylinus* (Zakaria and Nazeri, 2012), *K. nataicola, K. hansenii* and *K. swingsii* (Lisdayanti *et al*., 2006 and Castro *et al*.,2011). The cellulose-producing bacteria are commonly found in natural sources such as flowers, vegetables, nuts, sugar cane and, in particular, rotten fruits (Park *et al.,* 2003).The production of BC depends on several factors such as bacterial species, cultivation media, cultivation method, temperature, pH, inoculum size, carbon source and nitrogen sources (Jung *et al*., 2005; Keshk and Sameshima, 2005; Chawla *et al*., 2009 and Zakaria and Nazeri, 2012).

Gamma radiation may cause some mutations to the microbial genes through the DNA repair mechanisms within the cells (Thacker, 1999). Microorganisms differ greatly in their resistance to radiation. The radiation resistance of a microorganism is measured by the so-called decimal reduction dose (D10 value), which is defined as the radiation dose (kGy) required to reduce the number of that microorganism by 10-fold (one log cycle) or required to kill 90% of the total number (Aquino, 2012). Such mutants with increased productivity can reduce the cost of the production process and may possess some specialized desirable characteristics (Karanam *et al*, 2008).

The main objective of this study is to maximize biocellulose productivity by *Komagataeibacter rhaeticus* K isolated from kombucha tea in Egypt through optimization of various parameters in the cultural conditions. Determinations of D10-value for bacterial cellulose producing isolate cells and enhancement the ability of *K. rhaeticus* K for cellulose Production by using random mutation effect by gamma radiation technique.

MATERIALS AND METHODS

Microorganism

Komagataeibacter rhaeticus K used in this study was isolated from kombucha tea in Egypt by using Hestrin-schramm (HS) liquid and agar media (Hestrin and Schramm, 1954) in Food Microbiology Lab, National Center for Radiation Research and Technology, Atomic Energy Authority. The isolated bacteria was identified by morphological, physiological and biochemical characteristics as well as by 16S rRNA gene sequence analysis.

Inoculum preparation

Inocula were prepared by transferring one colony of *K. rhaeticus* K from HS agar plate to sterile 50-ml conical flasks containing 10 ml HS liquid medium. The culture was incubated statically at 30^{o}C for 48h.

Cultivation and harvesting of BC

One ml of 48h fresh culture was added into a sterile 250ml conical flask containing 50ml liquid HS medium and incubated statically at 30^{o}C for 7 days. The white pellicle formed on the surface was harvested through filtration and purified by treating with

0.5 N NaOH at 90^{o}C for 1h to remove the bacterial cells and medium component and then rinsed with water three times. The BC pellicle was dried at 105^{o}C for 12 -24h, or until at constant weight (Suwannapinunt *et al*., 2007).

Fermentation

Hestern-Schramm (HS) broth medium composed of (g): glucose 20, peptone 5, yeast extract 5, Na_2HPO_4 2.7 and citric acid 1.15 in 1L distilled water (Hestrin and Schramm, 1954), Glucose ethanol medium (GEM) composed of (g): glucose 15, peptone 3, yeast extract 3, ethanol 5ml in 1L distilled water and sucrose acetic acid medium (SAM) composed of (g): sucrose 20, yeast extract 3, peptone 3 and acetic acid 3ml in 1L distilled water (Hanmoungjai *et al*., 2007) were used for the production of BC. One ml of 48h fresh HS culture was transferred into 50- ml conical flasks of each medium and incubated at 30^{o}C for 7 days. After cultivation period, BC was harvested, purified, washed and dried as previously mentioned.

Media optimization

Several factors (temperature, Incubation period, pH values, culture age, inoculum size, static and agitation culture, various carbon sources, various nitrogen sources as well as glucose and ethanol concentrations) were tested in sequence to determine the optimal conditions of culture medium for the production of BC. Incubation under different fermentation temperatures (15, 20, 25, 30, 35 and 40ºC) was tested first for 7 days of incubation. Then, different incubation times (from 1 day to 10) to establish the best incubation period. Medium acidity and alkalinity were conducted through different pH ranges from 3 to 8. For the determination of the best culture age, 1ml of freshly prepared culture was taken during different growth phases (24, 48, 72, 96 h) and transferred to the fermentation medium. Then different inoculum volumes (1, 2, 3, 4, 5% (v/v)) from the 48h freshly culture were used to identify the best inoculum size. The incubation under static and agitated fermentation was also tested. Different carbon (glucose, maltose, fructose, galactose, mannitol, melibiose, sucrose, mannose and trehalose) as well as nitrogen sources (yeast extract & peptone, beef extract, ammonium chloride and potassium nitrate) were tested.

After best carbon source was chosen, the test for its optimum concentration was followed at different concentrations [1-5% (w/v)]. Finally, ethanol was added to the modified medium at concentrations of 0.5-2.5% (v/v). On the other hand, one flask was left without addition of ethanol as a control. All the experiments were carried out into 250 ml conical flasks and incubated for the specific time period. After cultivation period, BC was harvested, washed and dried as previously mentioned.

Gamma radiation

To construct radiation response curve of bacterial isolate, test tubes containing 10ml of fresh HS culture were exposed to different doses of gamma radiation (0, 0.5, 1, 1.5 and 2.0 kGy). After irradiation, the tubes were ten-fold diluted, cultivated on HS agar media and incubated at 30ºC for 48h .The colonies were counted in each dish and the D10 value was calculated for bacterial cells. The D10 values for the isolate was determined from radiation dose response curves and from the regression linear equation $y = a + bx$ (Lawerence, 1971). For determination the effect of gamma ray on BC production, test tubes

containing 10ml of fresh GEM culture were exposed to low doses (0, 0.2, 0.4, 0.6 and 1.0kGy) of gamma radiation. After irradiation, one ml of each tube was cultivated on GEM agar medium and incubated at 30ºC for 48 h. Single colony from each dish was transferred into 50-ml conical flask containing 10 ml GEM liquid medium (pH 5), then 3 ml of 48h fresh culture was added into a sterile 250ml conical flask containing 50 ml GEM liquid medium (pH 5). The flasks were incubated at the optimized conditions. After cultivation period, BC was harvested, washed and dried and the effect of gamma ray on BC production was determined. Irradiation was carried out using cobalt- 60 irradiation source (Gamma Chamber 4000 India) located at National Centre for Radiation Research and Technology (NCRRT) – Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt. The irradiation dose rate at the time of experiment was 2.08 kGy/h.

Parameters of bacterial cellulose production

Productivity and conversion factor were calculated according to Vieira *et al*. (2013) using the following equations:

Productivity (g/d) = cellulose dry weight (g/l)/ fermentation time (d)

Conversion factor (g/g) = cellulose dry weight (g/l)/original sugar (g/l)

Statistical analysis

The experiments were carried out in triplicate, and results were reported as mean ± standard deviation values. Analysis of variance (ANOVA) of data was carried out using IBM SPSS version 22.0.

RESULTS AND DISCUSSION

Komagataeibacter rhaeticus K used in this work was isolated from kombucha tea in Egypt (Identification results not shown). Fig. (1) shows the results of BC dry weight (g/l), productivity (g dry BC/day) and conversion factor (g dry cellulose/g sugar) by *Komagataeibacter rhaeticus* K on three different media. It is obvious that the level of BC produced in the used media ranged from 1.4 to 4.0 g dry weight/l. These values are in accordance with the values obtained by Suwanporsi *et al.* (2013) who found that the isolate *Gluconacetobacter* isolate PAP1 gave 1.15g/L BC in HS medium and BC yield increased three fold (3.5g/l) when D-glucose in HS medium was replaced by D-manitol. However these values are higher than that obtained by El-Saied *et al*. (2008) who found that the maximum yield of BC by *Gluconacetobacter sub SP*. *Xylinus* (ATCC 10245) was 0.792 g/l in manitol medium and 1.045 g/l in corn steep liquor medium. Also, Coban and Biyik (2011) found that BC produced in HS broth by *Acetobacter pasteurianus* HBB6 was in the range of 0.007 to 0.04g/l and for *A. Lovaniensis* HBB5 was in the range of 0.006 to 0.035g/l. However, our results are lower than that reported by Gayathry and Gopalaswamy (2014) who found that the yield of BC produced in HS medium by *A. Xylinum (sju-1)* was 11g/l. From our study it is obvious that GEM medium gave the highest BC production (4.0±0.3g/l) followed by HS medium (2.4±0.11g/l). The lowest BC production (1.14±0.3g/l) was recorded in SAM medium. The highest production of BC in GEM medium could be attributed to the presence of ethanol in this medium

which resulted in increasing the BC production by removing cellulose negative phenotypes from the population (Park *et al*., 2003). It is a well-documented fact that production of biocellulose is influenced by the presence of ethanol (Saxena and Brown, 2005).

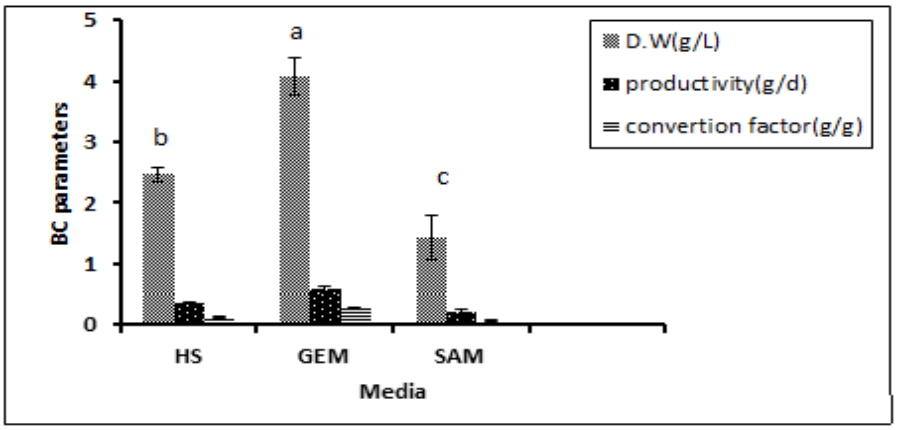

Fig. (1): Effect of Different media on BC production by *K. rhaeticus* K
- Data are expressed as mean values ± standard deviation (n = 3)
- Columns with different superscripts (a-c) are significantly different (p<0.05)

The influence of incubation temperature on BC production was shown in Fig. (2). There was no BC production by *K. rhaeticus* K recorded in liquid GEM medium at 15°C and at 40°C. The maximum production (4.1±0.32g/L) was found at 30°C. Many investigators found that the optimum growth temperature for biocellulose production was observed at 30°C (Son *et al*., 2001; Pourramezan *et al*., 2009; Çoban and Biyik, 2011; Zkaria and Nazeria, 2012 and Abd-elhady *et al*., 2015).

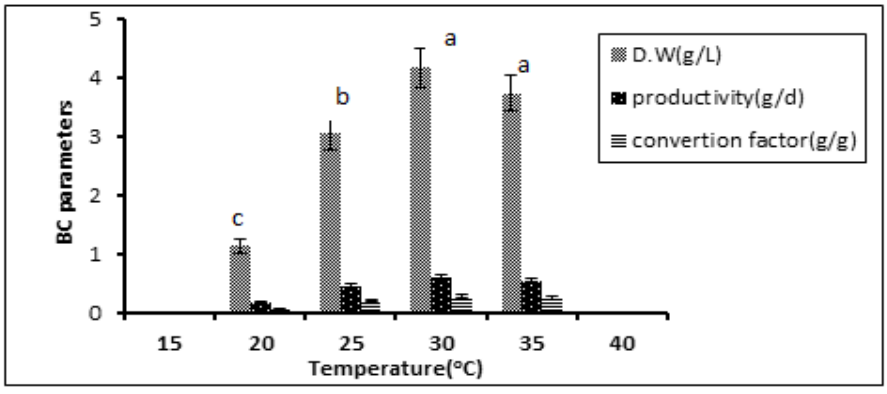

Fig. (2): Effect of different incubation temperatures on BC production by *K. rhaeticus* K
- Data are expressed as mean values ± standard deviation (n = 3)
- Columns with different superscripts (a-c) are significantly different (p<0.05)

Fig. (3) Represents the influence of period on BC production. BC was significally increased as incubation period increased. The maximum production of BC was recorded at 7 days of incubation. After 7 days there was no significant increase in BC production. These results are almost similar to that obtained by Surma-Ślusarska *et al.* (2008) who reported that the greatest increase in the weight of BC obtained from *Acetobacter xylinum* took place after 7-8 days. Panesar *et al.* (2009) also reported that the maximum production of BC (1.6g/L) by *Acetobacter aceti* MTCC 2623 was obtained after 7 days incubation period. El-Saied *et al*. (2008) found that the rapid enhancement of BC production by *Gluconacetobacter Xylinus* (ATCC10245) in corn steep liquor medium as the incubation period increased up to 7days reaching a maximum BC production of about 3g/L.

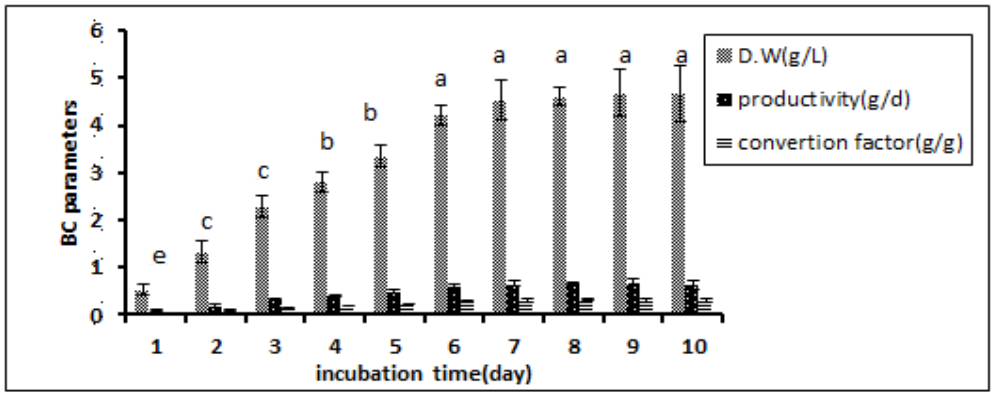

Fig. (3): Effect of different incubation time on BC production by *K. rhaeticus* K

- Data are expressed as mean values ± standard deviation (n = 3)

- Columns with different superscripts (a-e) are significantly different ($p<0.05$)

The pH value of growth medium plays an important role in the production of BC. Fig. (4) indicates that *K. rhaeticus* K under investigation could produce BC in a wide range of pH value. The highest biocellulose production (4.2g/L) was recorded at pH 5, while the lowest production (1.26g/L) was recorded at pH 8. These results are linked with those obtained by Zakaria and Nazeri (2012) who found that the pH 5.5 was the optimum for BC production by *A. Xylinum.* Fontana *et al.* (1990) found that the optimum pH range for cellulose production by *A. Xylinum* was 4 to 6, while Galas *et al.* (1999) demonstrated pH 4 to 7 as optimum for BC production by *A. Xylinum*. Masaoka *et al*. (1993) used pH 6 as optimum for BC production by *A. xylinum*. Verschuren *et al*. (2000) reported pH 4.0 and 5.0 to be ideal for the development of BC obtained from *A. xylinum*.

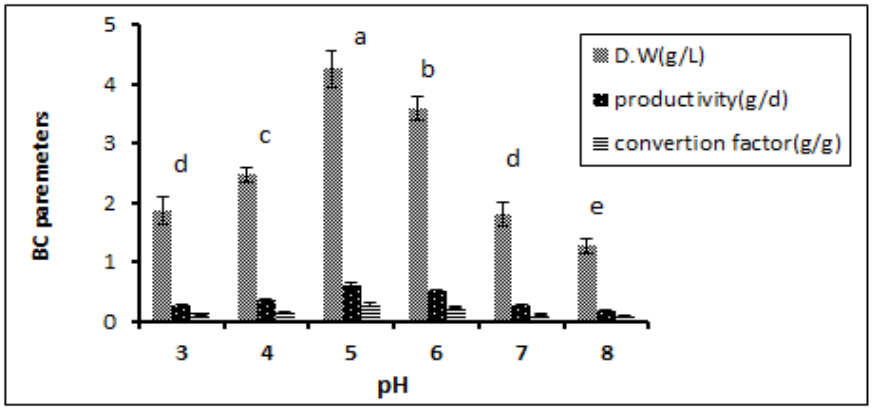

Fig. (4): Effect of different initial pH values on BC production by *K. rhaeticus* k strain

- Data are expressed as mean values ± standard deviation (n = 3)

- Columns with different superscripts (a-c) are significantly different (p<0.05)

The influence of culture age and size on the production of *K. rhaeticus* k grown on GEM medium at 30°C for 7 days under static condition is shown in Figures (5&6). Fig. (5) Indicates that BC production reached its maximum (4.4g/L) with 48h cultural age, then decreased after 72 and 96h.Fig. (6) Shows that there was a fluctuation of the cultural size results. However, the maximum dry weight (4.6g/L) of BC was found at 6% (v/v) of inoculum size.

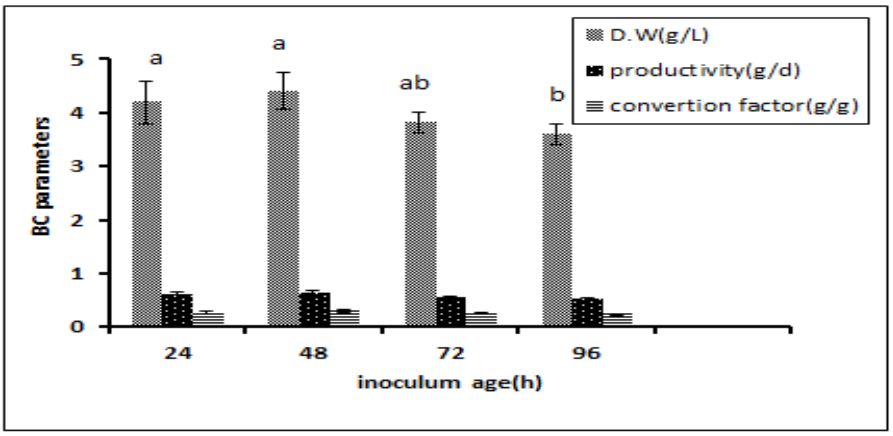

Fig. (5): The effect of different inoculum ages on the production of BC.

- Data are expressed as mean values ± standard deviation (n = 3)

- Columns with different superscripts (a-b) are significantly different (p<0.05)

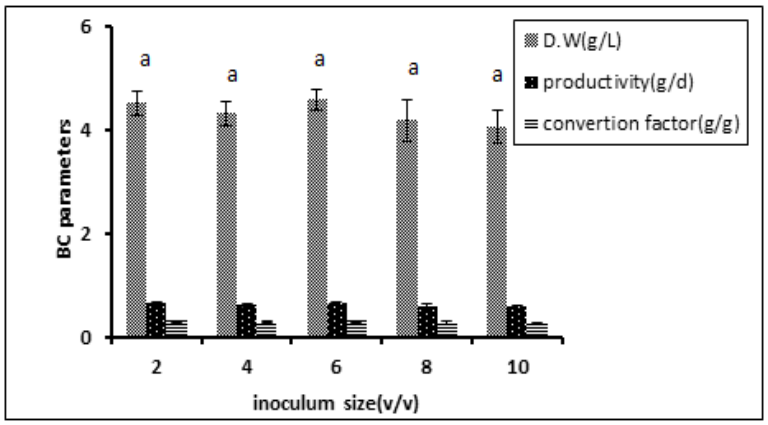

Fig. (6): The effect of different inoculum volumes on the production of BC
- Data are expressed as mean values ± standard deviation (n = 3)
- Columns with similar superscripts (a) aren't significantly different ($p<0.05$)

BC can be produced under agitation or static culture condition. In this experiment the cultivated culture was incubated at 30°C under static and agitation (150 rpm) condition for 7 days. Fig. (7) shows that incubation under static conditions gave higher BC yield (4.7±0.57g/L) than incubation under shaking conditions which only gave 2.5g/L. Many researchers have found that more BC was produced in static than that in agitated one. The problem in agitated culture is formation of cellulose- non producing mutants, which give low BC concentrations and a BC with no uniform structures (Valla and Kjosbakken, 1982 and Park *et al*., 2004). Culturing *G. hansenii* under agitated conditions resulted in the formation of cellulose negative (Cel^-) phenotypes, which become enriched over time in comparison to wild type phenotypes, resulting in low cellulose production (Kim *et al*., 2007).

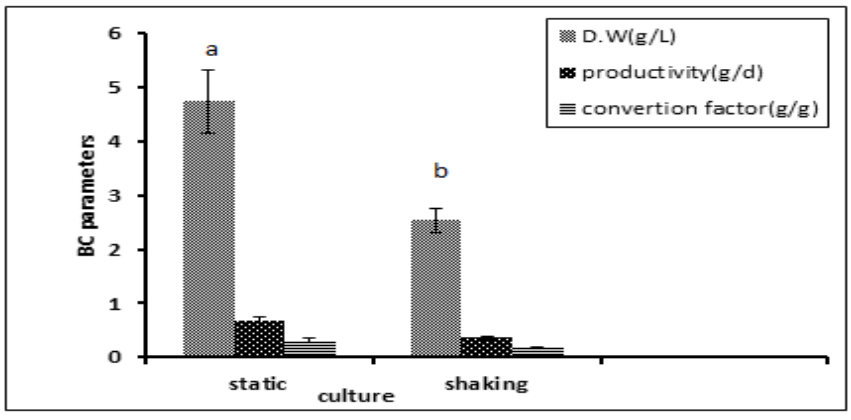

Fig. (7): Comparison between agitated and static culture for BC production by *K. rhaeticus* K
- Data are expressed as mean values ± standard deviation (n = 3)
- Columns with different superscripts (a-b) are significantly different ($p<0.05$)

To optimize carbon source for maximum production of BC by *K. rhaeticus* K in GEM medium, different carbon sources were used at concentration of 1.5% (w/v). In this experiment, our culture showed its capability of utilizing a wide variety of carbon sources for BC production but at different levels. Table (1) shows that the highest BC yield (4.6 g/L) was recorded with glucose as carbon source followed by sucrose (1.0 g/L) and manitol (0.7 g/L). These results are in good agreement with the results of Jonas and Farah (1998), who reported that the maximum BC production was achieved by supplementing the medium by 2% (w/v) glucose. They added that glucose was selected as carbon source due to the cost of manitol. *G. persinamonis* produced 5.14 g/L of BC when glucose was provided as carbon source. Travatti *et al*. (2011) reported that *G. sacchari* isolated from kombucha gave the highest production (2.7 /L) of BC with D-glucose as carbon source. Many other investigators reported that glucose as carbon source gave the highest BC yield (Coban and Biyik, 2011 and Raghunathan, 2013).

Table (1): Effect of different carbon sources on bacterial cellulose production by *K. rhaeticus* K grown on GEM medium.

Carbon source	Dry wt(g/L)	Productivity(g/d)	Conversion factor(g/g)
Glucose	**4.6±0.23^{a}**	**0.6571±0.02^{a}**	**0.3066±0.01^{a}**
Sucrose	1±0.17^{b}	0.1457±0.02^{b}	0.068±0.01^{b}
Maltose	0.14±0.023^{e}	0.0209±0.003^{e}	0.0097±0.001^{e}
Fructose	0.45±0.07^{d}	0.0647±0.011^{d}	0.0302±0.005^{d}
Galactose	0.15±0.009^{e}	0.0215±0.001^{e}	0.01±0.0006^{e}
Mannitol	0.73±0.02^{c}	0.1047±0.003^{c}	0.0488±0.001^{c}
Arabinose	0.41±0.12^{d}	0.058±0.02^{d}	0.0271±0.008^{d}
Melibiose	0.2±0.011^{e}	0.0295±0.002^{e}	0.0137±0.0007^{e}
Trehalose	0.51±0.02^{d}	0.0723±0.003^{d}	0.0337±0.002^{d}

- Data are expressed as mean values ± deviation (n = 3)
- Columns with different superscripts (a-e) are significantly different ($p<0.05$)

Table (2) shows the effect of different organic and inorganic nitrogen sources on the production of BC by tested culture. Organic nitrogen sources gave cellulose production higher than inorganic sources. The highest BC yield (4.4±0.23g/L) was recorded when a combination of yeast extract and peptone were used followed by yeast extract alone and peptone alone. The lowest yield (1.1±0.23) was obtained when ammonium chloride was used. These results are in the line with Kim *et al*. (2006) who found that the addition of yeast extract gave the highest BC yield (4.49 g/L) by *Gluconacetobacter sp.* RKY5 were Pourramezan *et al*. (2009) which obtained BC concentrations of 11.65 g/L by *Acetobacter sp.*4B-2with 0.7% yeast extract and 0.9 % peptone.

Table (2): Effect of different nitrogen sources on bacterial cellulose production by *K. rhaeticus* K grown on GEM medium.

Nitrogen Source	Dry wt (g/l)	Productivity (g/d)	Conversion factor(g/g)
Yeast & Peptone	**4.4±0.23[a]**	**0.657±0.03[a]**	**0.306±0.01[a]**
Yeast extract	3.1±0.3[b]	0.43±0.04[b]	0.204±0.02[b]
Peptone	3±0.2[b]	0.428±0.03[b]	0.2±0.01[b]
Beef extract	2.2±0.23[c]	0.323±0.03[c]	0.151±0.01[c]
Potassium nitrate	1.6±0.11[d]	0.238±0.02[d]	0.111±0.008[d]
Ammonium chloride	1.1±0.23[e]	0.162±0.032[e]	0.075±0.02[e]

- Data are expressed as mean values ± standard deviation (n = 3)

- Columns with different superscripts (a-e) are significantly different ($p<0.05$)

From Table (1), it was clearly obvious that, glucose proved to be the most suitable carbon source for BC production. Thus, glucose concentration on the BC production was investigated. The highest bacterial cellulose yield (6.4±0.41) obtained at 5% glucose concentration (Fig.8). These results are similar with those obtained by Son *et al.* (2001) who found that the highest BC yield by *Acetobacter sp.* A9 was obtained at 4% (W/V) glucose concentration.

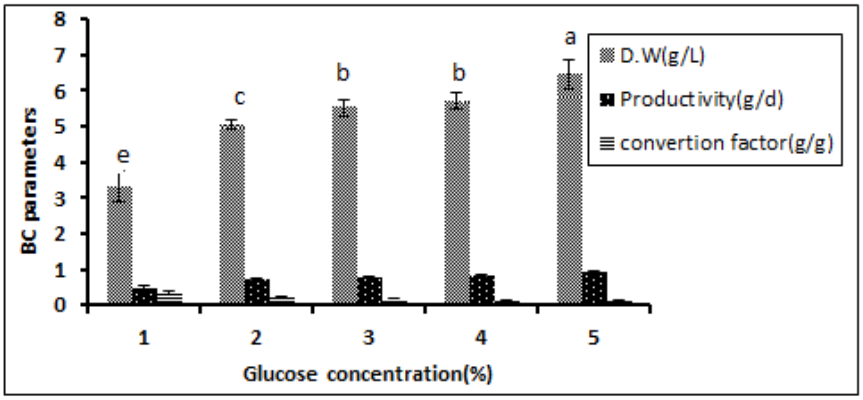

Fig. (8): The effect of different glucose concentration the production of BC
- Data are expressed as mean values ± standard deviation (n = 3)
- Columns with different superscripts (a-e) are significantly different ($p<0.05$)

The effect of different ethanol concentrations were represented in Fig (9). The highest BC yield (8.9±0.4) was obtained at 2 % (v/v) of ethanol which was about five times higher than that of media without ethanol. These results are in accordance with Son *et al.* (2001) who found that adding 1.4% g/L ethanol to 40 g/L glucose increased BC production to 15.2g/L, which was about four times higher than that of media without ethanol.

Some other investigators reported that when ethanol is used in combination with glucose the efficiency of cellulose production was enhanced in some strains of *G. hansenii* (Jung *et al*., 2005; Li *et al*., 2012; Masaoka *et al*., 1993; Park *et al*., 2003). The influence of ethanol on *G. hansenii* growth and cellulose synthesis have found ethanol to be effective at removing cellulose negative phenotypes from the population (Park *et al*., 2003).

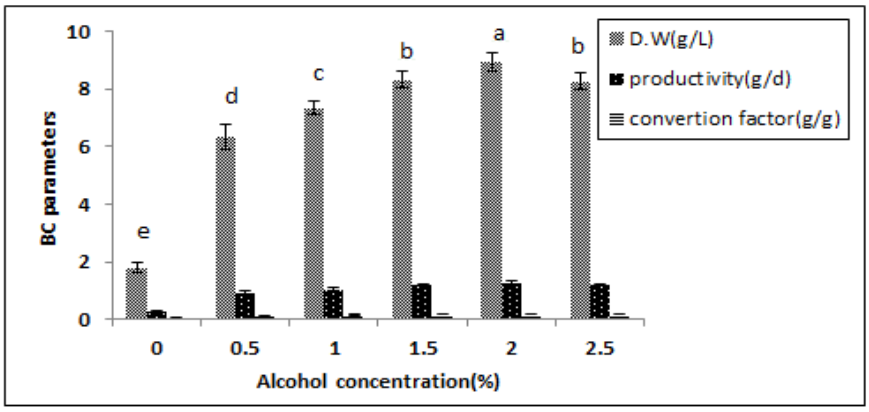

Fig. (9): The effect of different ethanol concentration on the productivity of BC
- Data are expressed as mean values ± standard deviation (n = 3)
- Columns with different superscripts (a-e) are significantly different ($p<0.05$)

Ionizing radiation can be used for induced mutation and enhancing the microbial productivity (Chuan-Xiao *et al.*, 2004). Ionizing radiation induce mutagenesis by generated reactive oxygen species (ROS) that react with DNA, RNA and their precursors lead to damage nucleic acids and nucleotides by detectable deletions, major rearrangements and into point mutation (Al-Sudany *et al.*, 2010). Fig. (10) shows the dose response curve of *K. rhaeticus* k which was 0.38 kGy. Fig. (11) shows the effective of different gamma radiation doses on bacterial cellulose production. The tested bacteria gave the highest yield of cellulose (9.3±0.4) when their cells are exposed to 0.4 kGy.

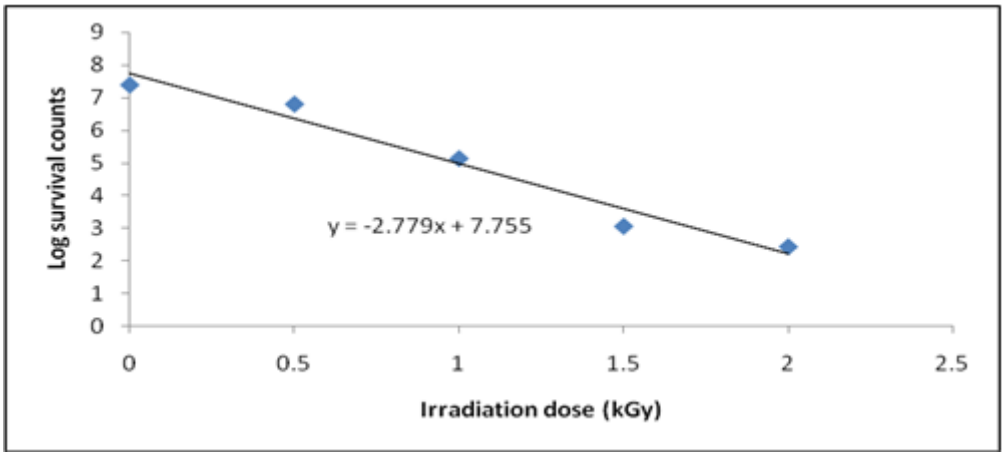

Fig. (10): Radiation dose response curve of *K. rhaeticus* K

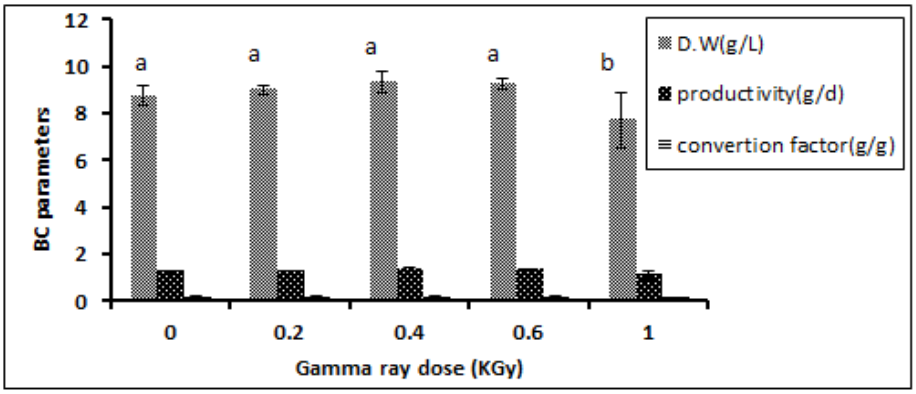

Fig. (11): Effect of different doses of gamma ray on the productivity of BC

- Data are expressed as mean values ± standard deviation (n = 3)

- Columns with different superscripts (a-b) are significantly different ($p<0.05$)

In conclusion, the optimized cultural conditions and gamma radiation dose for *K. rhaeticus* K were determined. The maximum BC dry weight yield (9.3±0.4g/L) by *K. rhaeticus* K was obtained in static culture under the optimized conditions compared with BC yield (4±0.3g/L) in normal standard medium. This means that the BC yield increase about132% under the optimized conditions. The results obtained from this study should help design better conditions for the production of cellulose by *K. rhaeticus* K isolate.

REFERENCES

Abdelhady, H.M.; Hassan, E.A.; Abd El-Salam, S.S. and Abdullah, S.M. (2015): Bacterial cellulose production as affected by bacterial strains and some fermentation conditions. Nature and Science, 13(3): 30-40.

Almeida, I.F.; Pereira, T.; Silva, N.H.C.S.; Gomes, F.P.; Silvestre, A.J.D.; Freire, C.S.R.; Sousa Lobo, J.M. and Costa, P.C. (2014)*:* Bacterial cellulose membranes as drug delivery systems: An in vivo skin compatibility study. Eur. J. Pharm. Biopharm., 86 (3): 332-336.

Al-Sudany, G.A.A.H.; Majeed, W.Z.; Jabbar, H. and Al-Aubeidi, A.R.A. (2010)*:* detection of gamma radiation effect induced by cobelt-60 on *Escherichia* coli cells. J. Al-Nahrain, 13: 129-133.

Aquino, K.A. (2012)*:* Sterilization by gamma irradiation edition. INTECH Open Access Publisher.

Basta, A. and El-Saied, H. (2009)*:* Performance of improved bacterial cellulose application in the production of functional paper. J. appl. Microbiol., 107 (6): 2098-2107.

Castro, C.; Zuluaga, R.; Putaux, J.-L.;Caro, G.; Mondragon, I. and Ganán, P. (2011)*:* Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp. from Colombian agroindustrial wastes. Carbohyd. Polym., 84 (1): 96-102.

Chawla, P.R.; Bajaj, I.B.; Survase, S.A. and Singhal, R.S. (2009)*:* Microbial cellulose: fermentative production and applications. Food Techn. Biotech., 47 (2): 107-124.

Chuan-Xiao, X.; An, X.;Li-Jun, W.; Jian-Min, Y.; Jian-Bo, Y. and Zeng-Liang, Y. (2004)*:* Comparison of base substitutions in response to nitrogen ion implantation and 60Co-gamma ray irradiation in *Escherichia coli*. Genetics and molecular biology, 27 (2): 284-290.

Çoban, E.P. and Biyik, H. (2011)*:* Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter lovaniensis* HBB5. Afr. J. Biotech., 10 (27): 5346-5354.

El-Saied, H.; El-Diwany, A.I.; Basta, A.H.; Atwa, N.A. and El-Ghwas, D.E. (2008)*:* Production and characterization of economical bacterial cellulose. BioResources, 3 (4): 1196-1217.

Fontana, J.; De Souza, A.; Fontana, C.;Torriani, I.; Moreschi, J.; Gallotti, B.;De Souza, S.; Narcisco, G.; Bichara, J. and Farah, L. (1990)*:* Acetobacter cellulose pellicle as a temporary skin substitute. Appl. Biochem. Biotech., 24 (1): 253-264.

Galas, E.; Krystynowicz, A.; Tarabasz-Szymanska, L.; Pankiewicz, T. and Rzyska, M. (1999)*:* Optimization of the production of bacterial cellulose using multivariable linear regression analysis. Acta biotechnologica, 19 (3): 251-260.

Gayathry, G. and Gopalaswamy, G. (2014)*:* Production and characterisation of microbial cellulosic fibre from *Acetobacter xylinum*. Indian J. Fibre Text. Res, 39: 93-96.

George, J. and Siddaramaiah (2012)*:* High performance edible nanocomposite films containing bacterial cellulose nanocrystals. Carbohydr. Polym., 87 (3): 2031-2037.

Hanmoungjai, W.; Chukeatirote, E.; Pathom-Aree, W.; Yamada, Y. and Lumyoung, S. (2007): Identification of acidotolerant acetic acid bacteria isolated from Thailand sources. Res. J. Microbiol., 2: 194-197.

Hestrin, S. and Schramm, M. (1954)*:* Synthesis of cellulose by *Acetobacter xylinum*. 2.

Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. Biochem. J. 58 (2): 345.

Jonas, R. and Farah, L.F. (1998): Production and application of microbial cellulose. Polymer Degradation and Stability, 59 (1): 101-106.

Jung, J.Y.; Park, J.K. and Chang, H.N.(2005): Bacterial cellulose productionby Gluconacetobacter hansenii in an agitated culture without living non-cellulose producing cells. Enz. Microb. Technol., 37 (3): 347-354.

Karanam, S.K. and Medicherla, N.R. (2008): Enhanced lipase production by mutation induced Aspergillus japonicus. Afri. J. Biotechn., 7 (12).

Keshk, S. and Sameshima, K. (2005): Evaluation of different carbon sources for bacterial cellulose production. African Journal of Biotechnology, 4 (6): 478-482.

Keshk, S. and Sameshima, K. (2006): The utilization of sugar cane molasses with/without the presence of lignosulfonate for the production of bacterial cellulose. App. Microbiol. Biotech., 72 (2): 291-296.

Keshk, S.M. (2014): Bacterial cellulose production and its industrial applications. J. Bioproc. Biotech., 4 (2): 1-10.

Kim, S.-Y.; Kim, J.-N.; Wee, Y.-J.; Park, D.-H. and Ryu, H.-W. (2006): Production of bacterial cellulose by *Gluconacetobacter sp*. RKY5 isolated from persimmon vinegar. App. Biochem. Biotech., 131: 705-715.

Kim, Y.-J.; Kim, J.-N.; Wee, Y.-J.; Park, D.-H. and Ryu, H.-W. (2007): Bacterial cellulose production by *Gluconacetobacter sp*. PKY5 in a rotary biofilm contactor. Applied biochemistry and biotechnology, 137 (1-12): 529-537.

Lawerence, C. E. (1971): Cellular radiobiology. William and Sons. Ltd., London.

Li, B.; Chen, X.; Ren, H.;Li, L.; Xiong, J.; Bai, J.; Chen, Y.; Wu, J. and Ying, H. (2012): Kinetic models of ribonucleic acid fermentation and continuous culture by Candida tropicalis no. 121. Bioproc. and biosystems engineering, 35 (3): 415-422.

Lisdiyanti, P.; Navarro, R.R.; Uchimura, T. and Komagata, K. (2006): Reclassification of *Gluconacetobacter hansenii* strains and proposals of *Gluconacetobacter saccharivorans* sp. nov. and *Gluconacetobacter nataicola* sp. nov. Int. J. Syst. Evol. Microbiol., 56 (9): 2101-2111.

Masaoka, S.; Ohe, T. and Sakota, N. (1993): Production of cellulose from glucose by *Acetobacter xylinum.* J.

Ferment. Bioeng,. 75 (1): 18-22.

Moon, R.J.; Martini, A.; Nairn, J.; Simonsen, J. and Youngblood, J. (2011)*:* Cellulose nanomaterials review: structure, properties and nanocomposites. *Chem. Soc. Rev.*, 40 (7):3941-3994.

Panesar, P.; Chavan, Y.; Bera, M.; Chand, O. and Kumar, H. (2009)*:* Evaluation of *Acetobacter* strain for the production of microbial cellulose. Asian J. Chem, 21 (10): 99-102.

Park, J.K.; Hyun, S.H. and Jung, J.Y. (2004): Conversion of *G. hansenii* PJK into non-cellulose-producing mutants according to the culture condition. *Biotechno.* Bioproc. Engin., 9 (5): 383-388.

Park, J.K.; Jung, J.Y. and Park, Y.H. (2003)*:* Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol. Biotech. Lett., 25 (24): 2055-2059.

Pourramezan, G.; Roayaei, A. and Qezelbash, Q. (2009)*:* Optimization of culture conditions for bacterial cellulose production by *Acetobacter* sp. 4B-2. Biotechnology, 8 (1): 150-154.

Raghunathan, D. (2013)*:* Production of microbial cellulose from the new bacterial strain isolated from temple wash waters. Int. J. Curr. Microbiol. App. Sci, 2 (12): 275-290.

Saxena, I.M. and Brown, R.M. (2005)*:* Cellulose biosynthesis: current views and evolving concepts. Annals of botany, 96 (1): 9-21.

Sherif, M. and Sameshima, K. (2005)*:* Evaluation of different carbon sources for bacterial cellulose production. African Journal of Biotechnology, 4 (6): 478-482.

Shi, Z.; Zhang, Y.; Phillips, G.O. and Yang, G. (2014)*:* Utilization of bacterial cellulose in food. Food Hydrocoll. 35: 539-545.

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*. Biotechnol. Appl. Biochem., 33 (1): 1-5.

Surma-Ślusarska, B.; Presler, S. and Danielewicz, D. (2008)*:* Characteristics of bacterial cellulose obtained from *Acetobacter xylinum* culture for application in papermaking. Fibers & Textiles in Eastern Europe, 4 (69): 108--111.

Suwannapinunt, N.; Burakorn, J. and Thaenthanee, S. (2007)*:* Effect of culture conditions on bacterial cellulose (BC) production from Acetobacter xylinum TISTR976 and physical properties of BC parchment paper. Suranaree

J. Sci. Technol, 14 (4): 357-365

Suwanposri, A.; Yukphan, P.; Yamada, Y. and Ochaikul, D. (2013)*:* Identification and biocellulose production of *Gluconacetobacter* strains isolated from tropical fruits in Thailand. Maejo. Int. J. Sci. Technol., 7 (1): 70-82.

Thacker, J. (1999)*:* Repair of ionizing radiation damage in mammalian cells. Alternative pathways and their fidelity. Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie, 322 (2): 103-108.

Trovatti, E.; Serafim, L.S.; Freire, C.S.; Silvestre, A.J. and Neto, C.P. (2011)*:* *Gluconacetobacter sacchari*: an efficient bacterial cellulose cell-factory. Carbohyd. Polym., 86 (3): 1417-1420.

Valla, S. and Kjosbakken, J. (1982)*:* Cellulose-negative mutants of *Acetobacter xylinum*. Microbiology, 128 (7): 1401-1408.

Verschuren, P.G.; Cardona, T.D.; Nout, M.R.; De Gooijer, K.D. and Van den Heuvel, J.C. (2000)*:* Location and limitation of cellulose production by *Acetobacter xylinum* established from oxygen profiles. J. Biosci. Bioeng., 89 (5): 414-419.

Vieira, D.C. Moretti; Ishii, B.T.G.; Senna, M.; Penna, T.C. Vessoni (2013): Bacterial cellulose production by *Acetobacter xylinum* and *Saccharomyces cerevisiae* in green tea leaves and fruits juice medium. A. J. P. H. S., 692-698.

Yamada, Y.; Yukphan, P.; Lan Vu, H.T.; Muramatsu, Y.; Ochaikul, D.; Tanasupawat, S. and Nakagawa, Y. (2012)*:* Description of *Komagataeibacter* gen. nov., with proposals of new combinations (Acetobacteraceae). J. Gen. Appl. Microbiol., 58 (5): 397-404.

Zakaria, J. and Nazeri, M.A. (2012)*:* Optimization of bacterial cellulose production from pineapple waste: effect of temperature, pH and concentration. Engineering Conference "Engineering towards Change - Empowering Green Solutions" 10-12th July 2012, Kuching Sarawak

ARABIC SUMMARY

"تعزيز إنتاجيه السيليلوز الحيوي من خلال ضبط الظروف المزرعيه و أشعه جاما"

للسادة الدكاترة

محمد محمد رشدي - عباس احمد الغمري – علي أحمد حماد* - سلوي ابو النور* - ناصرحمدي محمد*

مــن

قسم النبات والميكروبيولوجي- كليه العلوم- جامعه الأزهر

*قسم الميكروبيولوجيا الأشعاعيه - المركز القومي لبحوث وتكنولوجيا الأشعاع - هيئه الطاقه الذريه

اجري هذا البحث بهدف ضبط الظروف المزرعية لإيجاد الظروف المثلي والتي بدورها تؤدي الي تعزيز إنتاجيه السيليلوز البكتيري (السيليلوز الحيوي) من بكتريا*كوماجاتيباكتر رايتكس* المعزوله من شاي الكمبوشا في مصر وتحديد الجرعة الإشعاعية التى تعمل على تعزيز الإنتاجية.

اثبتت النتائج أن بيئه جلوكوز- ايثانول السائله هي الأنسب لإنتاج السيليلوز البكتيري بواسطه هذه البكتريا وأنها تستطيع النمو علي مدي واسع من درجات الحراره (20-35°م) ودرجات اس هيدروجيني تتراوح ما بين (3-8). وبتعريض هذه السلاله لجرعات مختلفه من اشعه جاما وجد ان قيمه الانخفاض العشري لها 0.38 كيلو جراي. كما وجد أن تشعيع السلاله بجرعه قدرها 0.4 كيلو جراي ادي الي زياده إنتاجيتها من السيليلوز البكتيري مقارنه بتلك التي لم تتعرض للإشعاع. أثبتت النتائج أيضاً أن الظروف المثلي التي أدت الي زيادة الإنتاجيه كالاتي:

درجه حراره تحضين (30°م) ، مده تحضين (7 أيام) ، درجه اس هيدروجيني (5) ، مصدر كربوني (الجلوكوز 50 جم/لتر) ، مصدر نيتروجين (ببتون 3جم/ لتر مع مستخلص الخميره 3جم/ لتر) ، إضافه إيثانول الي بيئه التخمر (20مل/لتر) مع تشعيع السلاله بجرعه قدرها 0.4كيلو جراي واجراء التحضين تحت ظروف ساكنه . تحت هذه الظروف المثلي وصلت إنتاجيه البيوسيليلوز الي (9.3جرام وزن جاف لكل لتر من البيئه السائله) مقارنه بإنتاجيه قدرها (4جرام/لتر) تحت ظروف التخمر العاديه.