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Investigating the Feasibility of Applying Xanthan, Dextran, and Rhizobia Exopolysaccharides for Paper Reinforcement

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Abstract

A new process using microbial exopolysaccharides (**EPSs**) was developed recently for paper consolidation. This procedure is completely unique in that, besides inhibiting fungal growth, it also improves the mechanical properties of paper. In the present article, the potential of enhancing the mechanical properties of the treated papers is investigated. three microbial exopolysaccharides (xanthan, dextran, and rhizobia exopolysaccharides) were produced by continuous culture The maximum production of xanthan and dextran were obtained by adding a fresh medium in bioreactor at 182 ml/h (0.091 h-1 dilution rate) and 288 ml/h (0.144 h-1 dilution rate), respectively, while the maximum concentration of Rhizobial exopolysaccharides was recorded at 136 ml/h (0.069 h-1 dilution rate). Continuous cultivation of X. campestris, R. legumin Sarum and L. mesenteroides increased the xanthan, Rhizobial exopolysaccharides and dextran production 23.6, 46.5 and 16.2%, respectively, comparing with bioreactor as a batch culture. Tests were undertaken to evaluate improvements in mechanical properties induced by these microbial biopolymers on two types of papers and compared with eight commercial polymers used broadly in paper consolidation. It was found that the exopolysaccharides of xanthan tested acted as reinforcement agents when introduced in the paper, significantly increasing the mechanical properties in comparison with the commercially available polymers used in paper conservation. The effect was significant in different papers tested which demonstrated the protection and strengthening of brittle paper. In addition, a 1.5% concentration of Xanthan functioned as mold inhibitors; no visible fungal growth was recorded for 90 days in low humidity on Whatman paper treated with Xanthan.

Keywords: Microbial exopolysaccharides, continuous culture, impregnating agent, coating film and paper conservation

1. Note

"Monosaccharides, disaccharides, and polysaccharides are well established in scientific literature- for paper reinforcement" (El-Ashmawy et al. 1973; Mobarak et al. 1973; Fahmy et al. 2006; Fahmy and Mobarak, 2008; Fahmy, 2007; Fahmy and Mobarak, 2009; Fahmy and Mobarak, 2014; Fahmy, 2017; Fahmy and Mobarak, 2008; Fahmy and Mobarak, 22011; Fahmy et al. 2017).

2. Introduction

Microbial exopolysaccharides (EPS) such as xanthan, dextran, pullulan and gellan are becoming more important as new materials and ingredients for a variety of industrial applications as natural gums (Morris and Harding, 2009; Moscovici, 2015). Due to their excellent rheological properties, these biopolymers have commercial interest because of their unique chemical and physical properties, which include stabilizing, thickening, gelling, coagulating, suspending, film-forming, water retention and metal binding (Andrew and Jayaraman, 2022). These

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characteristics make them suitable for different industrial applications including detergents, textiles, adhesives, paper coating, food, and beverage applications as well as pharmaceuticals, cancer therapy, drug delivery, oil recovery, textile, agriculture, cosmetic industries, metal recovery in the mining industry and from industrial waste. (Ji et al. 2022; Zhou and Huang, 2023; Ates, 2015; Tang and Huang, 2022; UL Qader et al, 2006 and Bramhachari; Dubey, 2006). Historical papers, i.e., documents, manuscripts and early printed book pages especially that were manufactured after the eighteenth century and made from mechanical and chemical wood pulp, become brittle, fragile and suffer from remarkable weakness due to variable factors such as deterioration of cellulose fibers and other components of papers (Da Silva Borges *et al*, 2018) as well as degradation of lignin, bleaching of residual materials and other paper- making process. Paper damage may be caused by other external factors including elevated temperature, relative humidity as well as light exposure (Mohamed and Ali, 2017). Atmospheric gaseous pollutants, especially these of sulfur dioxide played a crucial role in the damaging and aging of paper (Vine and Hollinger, 1993).

There are various techniques that might be considered to consolidate brittle, degraded, and damaged paper objects. These techniques include washing, deacidification, introducing a secondary support, lamination resizing or impregnating with a material to strengthen the brittle paper (Sundholm and Tahavaninen, 2004; Zervos & Moropoulou, 2007). Consolidation and preservation decisions depend on many factors including the causes of damage and the state of the object itself (de Graaff, 1981).

In the present work, we compared between eight commercially available polymers that already known and used in various restoration methods and three microbial polymers prepared in our laboratory as conservating agents of deteriorated historical papers. The prepared exopolysaccharides were characterized and evaluated for conservation and strengthening of weak and brittle paper as impregnate agents. All of them were evaluated as impregnating agents on two different modern papers, Whatman filter paper and newsprint paper. The purpose of the treatment imparts the aged and damaged paper strength and obtains an object that is not only for storage or display, but also for use. The treated sheets should be unchanged in dimensions, colour, appearance, and surface morphology.

3. Materials and Methods

3.1. Microbial strains used.

Provided from Microbiology Department, while the fungal species Penicillium pinopbilum ATTC 9644, Aspergillus niger ATTC 9642 and *Chaetomium globosum* ATTC 6205 were obtained from Cairo MIRCEN, Faculty of Agriculture Ain Shams University. Cairo, Egypt. All strains were maintained at 5ºC and transferred monthly on fresh slants.

3.2. Media used.

Medium.1: - YM medium (Roseiro *et al*, 1992) was used for preservation and propagation of *Xanthomonas campestris* ATCC 13951.

Medium.2: - Garcia- Ochoa´s medium (Garcia-Ochoa *et al*, 1992) was used for xanthan production.

Medium3: - McCleskey medium **(McCleskey *et al*, 1947)** was used for preservation of *L. mesenteroides* and dextran production.

Medium4: - Yeast extract mannitol (YM) (Vincent, 1970) was used for preservation of *R. legumino Sarum* and Rhizobial exopolysaccharides production.

Medium.5: - Czapek´s medium (Difco Manual, 1977) was used for growing of fungi.

3.3. Standard inocula

Standard inocula of microbial cultures were prepared by transferring a loop of the tested cultures into 250-ml Erlenmeyer flasks containing 50ml of productive media (med.1 in case of X. campestris). The inoculated flasks were incubated on a rotary shaker at 150rpm for 24 h at 30°C (the flasks were incubated statically at 22°C for 24 h in case of L. mesenteroides). The content of these flasks was used as standard inocula (1 ml contained 5.0–7.0 x 107 viable cells) for all experiments.

3.4. Fermentation process

In the present work 3L dished bottom bioreactor Z6110/Coob (Cole-Parmer Instrument) was used, which consists of 3-liter vessel equipped with lip seal stirrer assembly, automatic pH controller, automatic dissolved O2 controller, CO2 controller, automatic

temperature controller, foam controller and multi-channel peristaltic pump (for feeding). The producing bacteria were grown in the bioreactor as batch and continuous cultivation.

3.5. Bioreactor as a batch culture

In this experiment the fermentation vessel containing 1900 ml productive medium was autoclaved at 121°C for 20 min (or at 100°C for 30 min and repeated three times, Arnold method, for McCleskey medium). The bioreactor was inoculated with 5% standard inoculums of tested strains. The final working volume was 2 liters. Initial pH was not controlled during the fermentation period. Temperature, dissolved O_2 and speed of agitation were kept at 30°C, 20 % of saturation and 750rpm, respectively, while, in case of *L. mesenteroides* they were kept at 22°C, 5 % and 100rpm, during batch cultivation. During fermentation, pH was automatically recorded, and samples (10-20 ml) were withdrawn from the culture (fermentation vessel) periodically till the end of the fermentation period. The samples were centrifuged at 14000*g* for 30min at 4°C after dilution with 50ml distilled water. The sediment (biomass) was washed twice with distilled water then dried at 70°C to constant weight. Polymer in samples was precipitated and determined. Growth and polymer parameters were calculated.

3.6. Bioreactor as a continuous culture

Continuous culture was carried out in the same bioreactor using 1900ml productive media. After sterilization, the media was inoculated by 5% standard inoculum. The final working volume was 2 liters. The culture in the vessel was allowed to grow as a batch culture for 120 h for X. campestris, R. legumino Sarum or 24 h for L. mesenteroides. After this period, fresh medium was pumped to the culture at different flow rates of 228, 240, 268, 288 & 308 ml/h to give 0.114, 0.124, 0.134, 0.144 & 0.154 h-1 dilution rates for L. mesenteroides and 122, 142, 162, 182 & 202 ml/h to give 0.061, 0.071, 0.081, 0.091 & 0.101 h-1 dilution rates for X. campestris or 78, 98, 118, 136 & 158 ml/h to give 0.039, 0.049, 0.059, 0.069 & 0.079 h-1 dilution rates for R. legumino Sarum, respectively. Cultivation of each dilution rate (steady state) was kept for 24h intervals. Samples were collected aseptically at each steady state to determine the biomass, polymers production and productivity.

3.7. Calculations

The specific growth rate (μ), doubling time (td) and flow rate of fresh medium (dilution rate) in continuous culture were calculated from the exponential phase according to Painter and Marr (1963). Numbers of generation and multiplication rate were calculated according to Stanier *et al* (1970). Biopolymers yield coefficient relative to cell dry weight ($y_{p/x}$) was calculated according to Grothe *et al* (1999). Biopolymers productivity (P) was calculated according to Wang and Lee (1997).

3.8. Biopolymers as impregnating agents

The microbial exopolysaccharides were compared with eight synthetic commercial polymers ex. Arabic gum, methylcellulose (tylose MH300), gelatine (sheets, pure grade Cod No 24343 Edwic. Pro.lab), maize starch, wheat starch, modified starch (Emsland Starka Gmbt 445g Emlichein), acrylic polymer emulsion (methyl methacrylate and butyl acrylate, Lascaux 498 HV) and carboxymethylcellulose sodium salt (Low Viscosity Edwic, CMC) as impregnating agents for paper. The paper substrates were Whatman paper No.1 and newsprint paper. We selected Whatman paper to simulate the effect on ancient papers in good condition and particularly to evaluate the effect of the treatment on the pure cellulose without the interference of other components and newsprint paper to simulate the effect on the most common law quality modern paper. All the impregnating agents mentioned above were dispersed in water at concentration 1.5 % (w/v) except methylcellulose used once in water only and another in water and alcohol 2:1. Whatman and newsprint paper sample were immersed in 1000 ml solution for 1-2 minutes, the paper sheets were placed between two sheets of washing support from synthetic woven materials (Remay), to facilitate the movement of paper sheets from the solution, Then the paper samples were transferred to a flat surface on a blotting paper allow the paper to dry in the appropriate manner to avoid any undesirable impression.

3.9. Aging of Samples

To evaluate the behavior of the test materials over time, it was decided to carry out artificial aging on treated and untreated samples Following the standard ISO 5630-5. The samples were exposed to 100°C±5

and less that 10% relative humidity in a laboratory oven for 14 days. then removed and conditioning at 24°C ±1 and 45% ±2 RH) for 24h.

3.10. Testing

After conditioning, the samples were subjected to mechanical and morphological tests. They are tested before and after accelerated aging with the following methods:

- A piece of 2.5 cm for each type of paper (Whatman paper, newspaper) were weighed up to constant values before and after each application.
- Tensile strength in machine and cross direction was obtained by (Tinius Olsen, Tensile Tester), according to standard EN ISO 13934-1; 1999 Maximum Force& Elongation- strip method. A set of 10 paper strips (110 × 20 mm size).
- Tear strength in machine and cross direction

Was carried out using tensile testing machine (Tinius Olsen) with a span distance of 50 mm and a crosshead speed of 500mm/min. the torn length of the specimen is 30mm, and the time needed for testing was 7.2s. (Yamauchi and Tanaka; 2002)

- Scanning electron microscopy (SEM)

Changes in the surface morphology of the untreated and treated samples were observed using SEM instrument, FEI Quanta 3D 200i, was operated under the following conditions: low vacuum for acceleration voltage 20KV and large field detector with working distance from 16.4 to 19 mm.

3.11. Growth of fungi

This experiment has been constructed to compare the treated papers with purified microbial exopolysaccharides and CMC as fungal growth barrier. microbial polymers-treated paper samples (3cm x 3cm, each) followed by standard concentration of CMC (1.5 %) were sterilized with ethylene oxide and inoculated with standard fungal spore suspension (1000 spore/ml) of Penicillium pinopbilum ATTC 9644, Aspergillus niger ATTC 9642 and Chaetomium globosum ATTC 6205 (Fausta et al, 1996). The samples were incubated for 90days (Petri dishes containing 5ml solution) at a constant temperature of 25°C at different values of relative humidity (R.H) between 100% and 43.16% as follow:

R.H= 100% with distilled water
R.H= 93.58 ± 0.55 % with KNO_3
R.H= 84.34 ± 0.26 % with KCl
R.H= 75.29 ± 0.12 % with NaCl
R.H= 52.89 ± 0.22% with $Mg(NO_3)_2$
R.H= 43.16 ± 0.39% with K_2CO_3

Microscopic examinations were made periodically. The scale below was used to evaluate the rate of fungal growth (Table 1).

Table 1 the scale that was used to evaluate the rate of fungal growth.

The range of attack	Classification	Description
Nothing	0	No visible attack
Thrifty	1	Small, or very widely dispersed, growth
Moderate	2	Intermittent or weakly dispersed attack
Abundant in patches	3	Substantial attack in patches
Generally abundant	4	General attacks over the entire surface or substantial attacks in patches, between the spots attacks occur in minor extent
Very abundant	5	Heavy substantial attacks over the entire material

3.12. Biopolymers determination

Xanthan, Rhizobial exopolysaccharides and dextran in supernatants were precipitated and determined as dry weight (gl^{-1}) according to methods recommended by Cadmus et al (1978), Wang et al (1999) and Shamala & Prasadit (1995), respectively.

4. Results and discussion

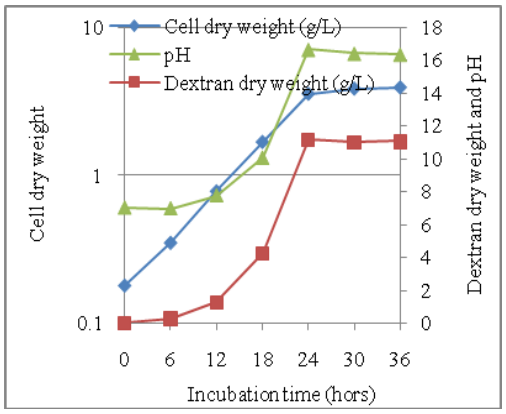
4.1. Biopolymers production and bacterial growth

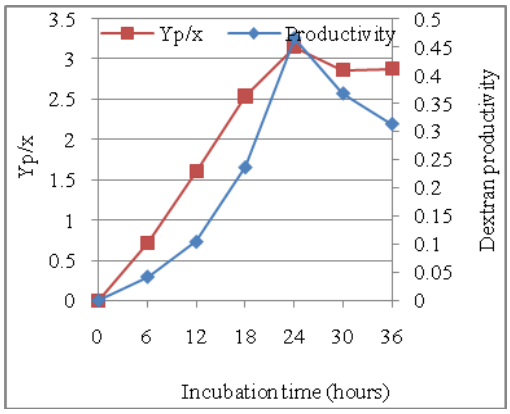
Data illustrated in Figure 1 illustrated characteristic fermentation profile of X. campestris, R. legumino Sarum and L. mesenteroides when grown on the production media. This data indicated that the first two strains grew exponentially during the first 48h of incubation and gave the highest cell dry weight (4.22 and 2.71 gl^{-1}) after 144 and 98h incubation by X. campestris and R. legumino Sarum, respectively. No great variation in the pH values was observed (5.4 to 6.1) at the end of fermentation period. The growth parameters of X. campestris during exponential phase were 0.071 h^{-1}, 9.76 h, 4.29 and 0.10 for specific growth rate, doubling time, number of generations and multiplication rate, respectively. Whereas the growth parameters of R. legumino Sarum were 0.049 h^{-1}, 14.14 h, 3.39 and 0.07, respectively. With respect to

biopolymers production, data also indicated that xanthan produced by X. campestris increased gradually during exponential growth phase and exhibited the highest values after 120h incubation (11.58 gl^{-1}, 2.75 and 0.098 gl^{-1} h^{-1} expressed as xanthan dry weight. xanthan yield coefficient relative to biomass ($Y_{p/x}$) and productivity, respectively). After 120h fermentation period, a slight decrease in xanthan production was noticed up to 144h. This may be due to hydrolysis during the stationary phase, suggesting critical importance of timing the harvest. While Rhizobial exopolysaccharides was not produced in the first 24h of incubation time. The highest polymer parameters were noticed after 120h (3.45 g/1, 1.28 and 0.029 g/1 h/1 for polymer dry weight, $Y_{p/x}$ and polymer productivity, respectively). On the other hand, the production of dextran by L. mesenteroides ATCC 17697 on production medium was started after 6h (in exponential phase) and continued during stationary phase with values of 11.15 gl-1, 3.14 and 0.465 g/1 h/1 for dextran dry weight, dextran yield coefficient relative to biomass (Yp/x) and productivity, respectively. These results agree with the results obtained by Sutherland (2002) who reported that most microbial exopolysaccharides were produced during exponential growth phase and continued until stationary phase.

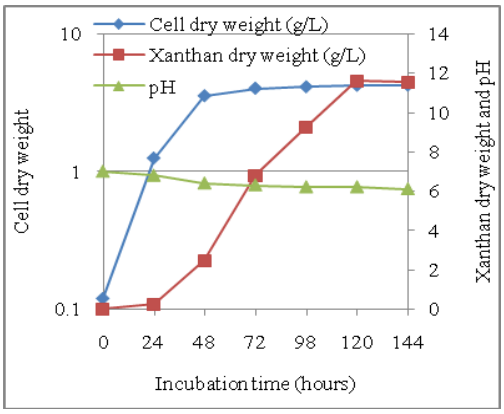
4.2. Polysaccharides production by continuous culture

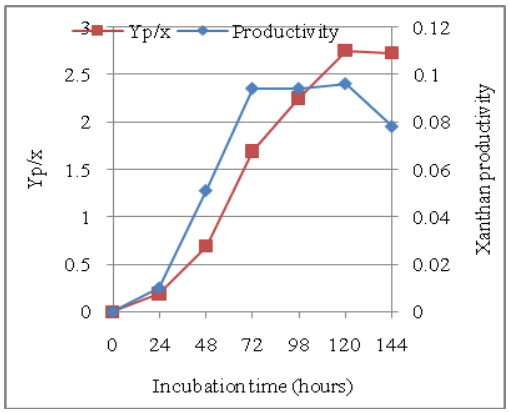
Continuous cultivations were carried out as previously described (materials and methods). The growth was studied at different dilution rates (different steady states) and growth parameters were calculated. The yield of biopolymers and their productivity were expressed as a function of dilution rates. The production medium was added to the
bioreactor at different flow rates ranged from 122 to 202ml hr-1, 78 to 158 and 228 to 308 ml h-1after 120h of incubation as a batch culture for X. campestris, R. leguminosarum and after 24h of incubation for L. mesenteroides, respectively. Results in Figure 2 showed that the change in the dilution rates caused a change in microbial biopolymers yield and productivity in different steady states. At steady state dilution rates of 0.114, 0.124, 0.134 and 0.144 h^{-1} of *L. mesenteroides* growth, dextran production and productivity remained constant for 24h in each dilution rate, while washing out was observed at 0.154 h^{-1} dilution rate. It is interesting to notice that the mean values of the highest amount of cell dry weight (3.75 gh^{-1}) was achieved at 0.144 h^{-1} dilution rate. Productivity and Yp/x ranged from 0.232 to 0.27 g l^{-1} h^{-1} and 3.14 to 3.77 respectively, for steady state levels from 0.114 to 0.144 h^{-1}. At 0.154 h^{-1} dilution rate, where no steady state was observed, the cells outlet decreased from 3.75 to 2.75 gh^{-1} during 24 h of incubation. Results also showed that the cells outlet dextran dry weight, dextran productivity and Yp/x increased with increasing of dilution rate, reaching their maximum at 0.144 h^{-1} dilution rate being 3.75 g h^{-1}, 12.96 g h^{-1}, 0.27 g l^{-1} h^{-1} and 3.46, respectively, and then decreased at 0.154 h^{-1} dilution rate. Therefore, it is concluded that the maximum dilution rate can be used for *Leuconostoc mesenteroides* strain is 0.144 h^{-1} which gives the maximum yield of dextran. While washing out was observed at 0.091h^{-1} dilution rate in case of *X. campestris*. The maximum cells production, xanthan concentration, xanthan productivity and Yp/x increased with increasing of dilution rate, reaching their maximum at 0.091 h^{-1} dilution rate with values of 3.95 g h, 13.12 g h, 0.096 g l^{-1} h^{-1} and 3.49, respectively, and then decreased at 0.101 h^{-1} dilution rate. In case of exopolysaccharides production by *R. leguminosarum* cells production, polymer concentration, polymer productivity and Yp/x increased with increasing of dilution rate, reaching their maximum at 0.069h^{-1} dilution rate (3.45 g /1, 4.99 g/1, 0.035 g l^{-1} h^{-1} and 1.45, respectively), and then decreased at 0.79h^{-1} dilution rate. Generally, it could be concluded from the previous results that continuous cultivation of *X. campestris*, *R. leguminosarum* and *L. mesenteroides* increased xanthan, Rhizobial exopolysaccharides and dextran production (23.6, 46.5 and 16.2 %, respectively) in comparison to bioreactor as a batch culture.



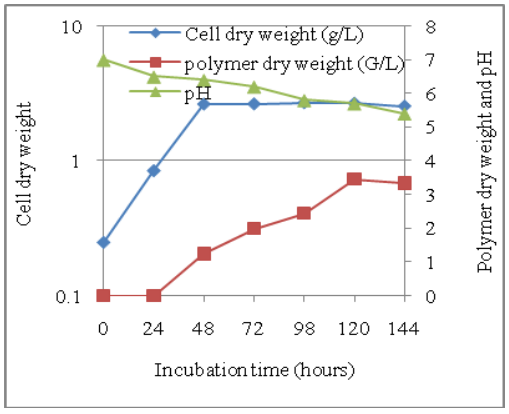


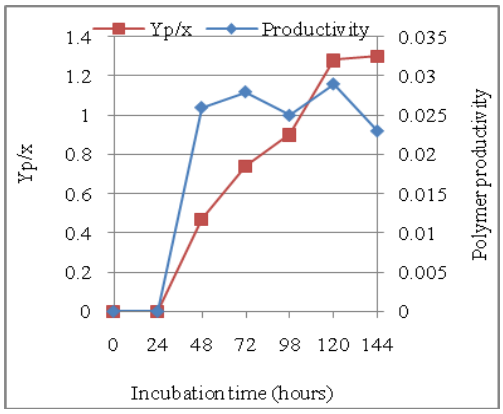
Dextran





Xanthan





Rhizobial exopolysaccharides

Figure 1: Growth of *L. mesenteroides, X. campestris* and *R. legumino Sarum* and microbial exopolysaccharides production on production media using bioreactor as a batch culture.

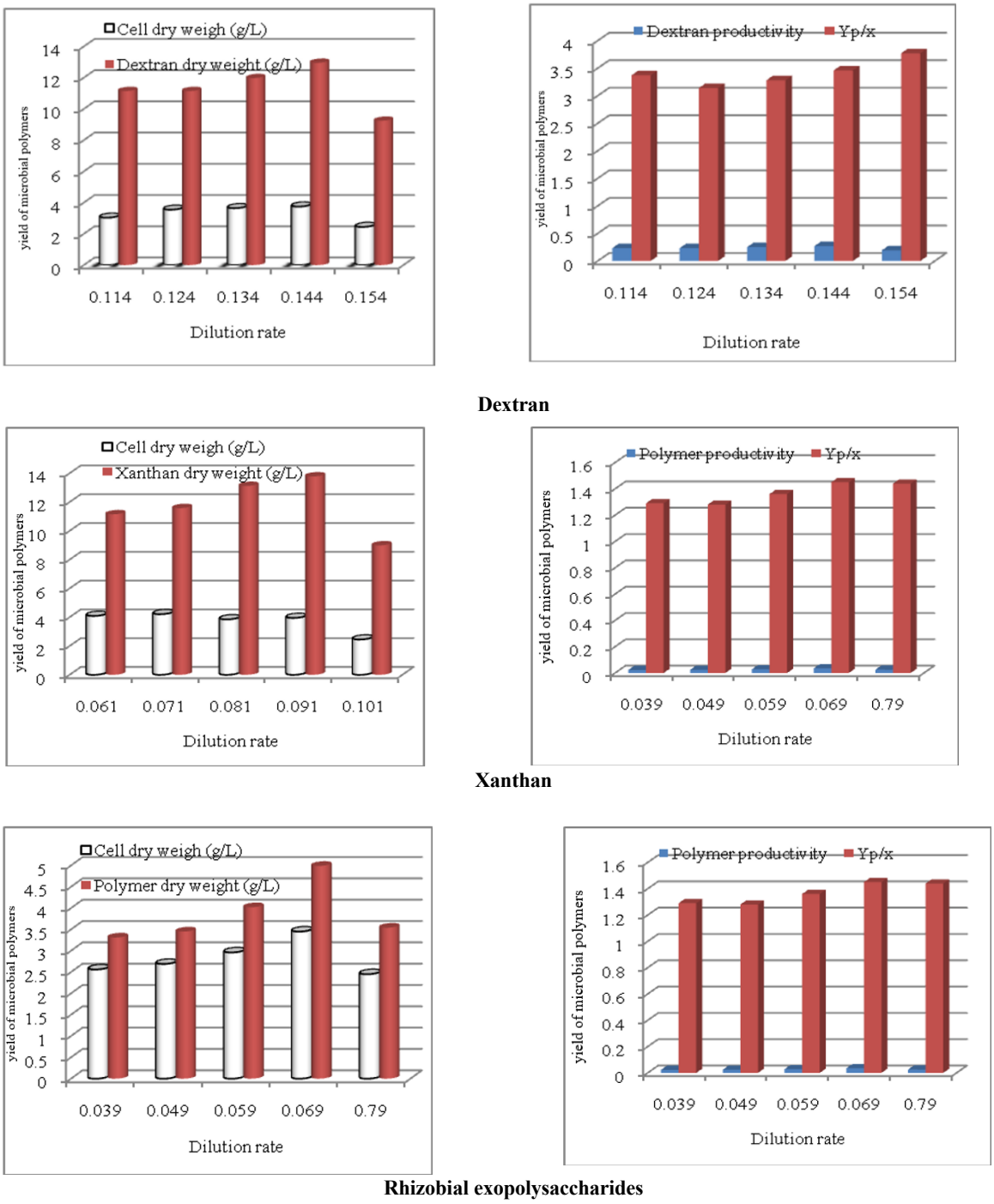


Figure 2: Growth of *L. mesenteroides, X. campestris* and *R. leguminosarum* and microbial exopolysaccharides production on productive media using bioreactor as a continuous culture.

4.3. Properties of impregnating papers

4.3.1. weight of paper

The samples prepared as described in section 2.8 showed an increase in weight after applying the different polymers. An increase in weight of about 10-15% for both unaged and aged papers occurred for

samples treated with Exopolysaccharides polymers; the same occurred for the commercially polymers with an increase of about 19- 21% in weight; but these samples are more stiffened than the samples treated with EPSs polymers.

4.3.2. Mechanical strength

4.3.2.1. Tensile strength

The results for treated paper samples at 1.5% concentration of impregnating agents for Whatman and newsprint samples, before and after aging (dry-oven aged up to 14 days) are reported in Figure 3 as the average of 12 measurements. As expected, there was a measurable increase in tensile strength for all treatments compared with untreated papers. The increment order of tensile strength after treatment was dextran > methylcellulose (W+ Eth) > maize starch > Acrylic polymer > CMC > gelatine > wheat starch. Xanthan and Rhizobial polymer and Arabic gum gave scarce strength compared with untreated paper, showing the lowest values. The other products showed intermediate values. After aging, tensile strength decreased in all the treatments, but retained greater than the untreated samples (control). There was discernible increase in many treatments. The decrease in tensile strength of aged samples clearly indicates the degradation reactions which take place as a result of cross- linking and decomposition.

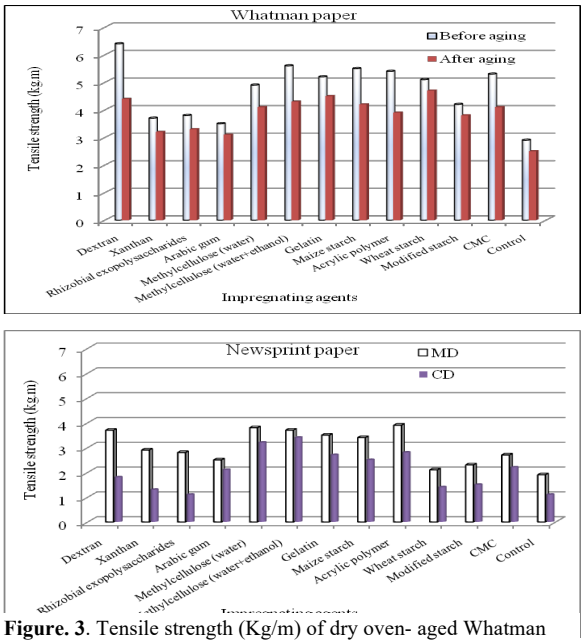


Figure. 3. Tensile strength (Kg/m) of dry oven- aged Whatman and Newsprint paper treated with different impregnating agents.

4.3.2.2. Tearing resistance

Figure 4 showed the tearing resistance of treated and untreated paper samples as well as dry oven-aged up to 14 days. There was a great increase for the Methyl-cellulose-treated samples with tylose MH300, Acrylic emulsion and carboxymethyl cellulose. The general behaviour of the treated paper subjected to tearing resistance tests closely parallels to results obtained for tensile strength, while the differences between the various treatments are less obvious in tear resistance than those obtained by tensile strength measurements. For both treated papers the tear resistance dropped after the accelerated aging but maintaining more height values than the untreated samples.

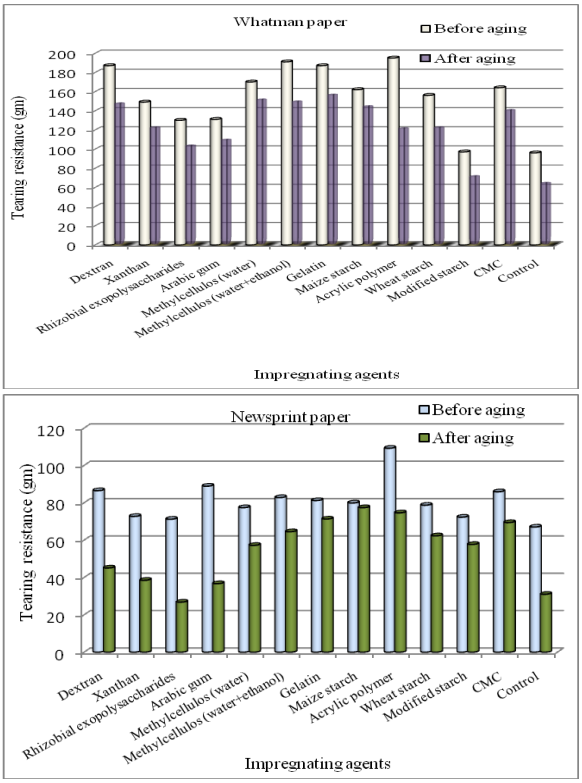


Figure. 4. Tearing resistance (gm) of paper treated with impregnating agents before and after aging.

4.3.3. Scanning electron microscopy (SEM)

Surface morphology changes of Whatman papers connected to application with the impregnating agents have been evaluated by Scanning electron microscopy (SEM) as illustrated in Figure 5. Each paper specimen was cut at random from the sample sheets. The surface morphology for each sample examined and compared with the control sample to check changes in the paper morphology. Figure 5 showed the surface morphology sheets with scanning electron microscopy, in general untreated sample (control) has more surface relief.

Xanthan agent does not produce any clear surface, the surface morphology is similar to control, panel the fibrous mat of paper remains porous. The treatment with dextran and Rhizobial polymer produced not significantly changes in the paper surface. When Arabic gum and acrylic emulsion are used, the surface looks slightly transparent. The surface of the paper treated with tylose (in water) does not show any special morphological changes. But when tylose is used with alcohol the surface has some opening, it may be an effect caused by alcohol which used as a solvent. Gelatine forms a network structure over the paper surface. All kinds of starch (maize, wheat, and modified starch) cover the surface of paper with film containing false dark holes in the paper structure that made the paper looks slightly opaquer. CMC forms a skin like layer over the paper surface and the surface holds a minor crack. From these experiments it is concluded, the impregnating agents are very well suitable for strengthening brittle and damaged paper. The paper gained satisfactory physical properties due to treatment with impregnating agents which added hydrogen bonds that leads to stronger Fiber to Fiber bonding. Tylose MH300, 1.5% (in water and alcohol) appeared to be the best and Arabic gum 1.5% to be the worst. There is no great difference between the tested polymers. All the materials have a positive effect on the aging properties of tested paper under the above-mentioned conditions.

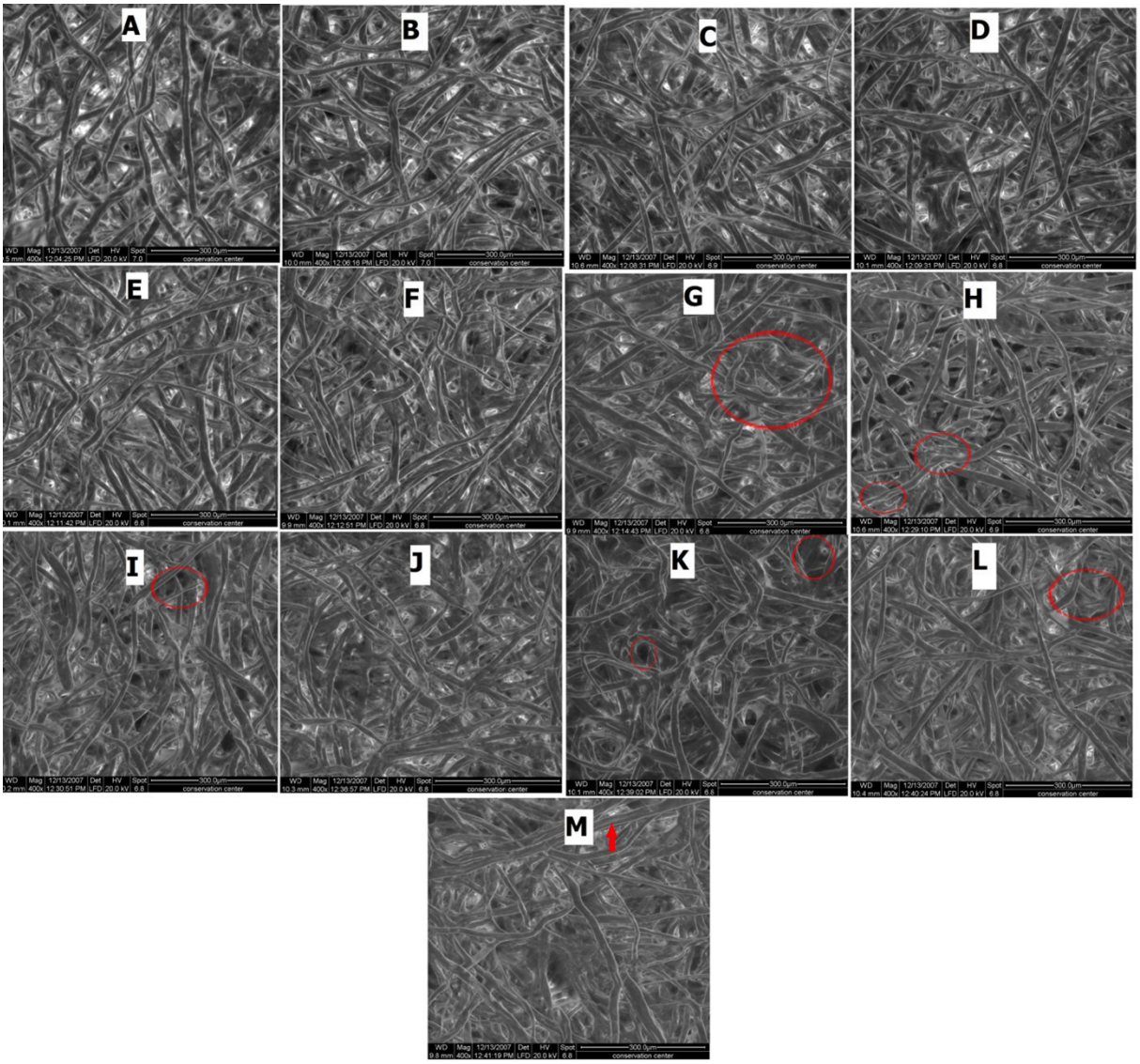


Figure. 5. Study of the surface morphology sheets with scanning electron microscopy (SEM). A: Control, B: Xanthan, C:Dextran, D:Rhizobial polymer, E:Arabic gum, F:Methylcellulose (in water), G: Methylcellulose (in water and alcohol), H: Gelatine, I:Maize starch, J:Acrylic Polymer, K:Wheat starch, L:Modified starch, M:CMC

4.4. Fungal growth on treated papers

The results in Figures 6 ,7 and 8 described the growth of different fungal genera on Whatman paper and newspaper treated with the same concentration of microbial exopolysaccharides or CMC as commercial synthetic polymer. The data clearly showed that the fungal growth was not recorded on Whatman paper treated with polymer solutions during the first 30 days of incubation except strife or very widely dispersed growth of *Aspergillus niger* and *Chaetomium globosum* on paper treated with Rhizobial exopolysaccharides at 84.34 to 100 % relative humidity and *Penicillium pinopbilum* at 93.58 to 100%. Also, the degree of fungal growth was higher on paper treated with Rhizobial exopolysaccharides followed by dextran. Xanthan and CMC solutions functioned best as mould inhibitors during the first 30 days of incubation at low relative humidity. Also, no visible fungal growth was recorded on Whatman paper treated with 1.5 % xanthan during 90 days at low relative humidity, where the first mentioned functioned best overall. The dextran and Rhizobial exopolysaccharides caused greater growth of fungi compared to the samples treated with xanthan or CMC and functioned therefore worse (as shown in Fig.5). The reason why the Rhizobial exopolysaccharides solution gave such bad results may be due to the simple chemical structure of exopolysaccharides of R. *legumino Sarum* and can be use as nutrients for fungi growth and reproduction.

On the other hand, all fungal genera observed visible growth on newsprint paper treated with Rhizobial exopolysaccharides or dextran solutions at all relative humidity except at 43.16 %. Likewise, xanthan was more resistance for hydrolysis by fungi genera used, whereas it delayed the fungi growth after 45 days of incubation.

The solution containing 1.5 % xanthan had the best properties of inhibiting fungi. This can depend on that there was no nourishment for the microorganisms. Another explanation can be that the coating provided the best barrier properties against moisture and oxygen that is necessary for mold growth. Also, it should be mentioned that the sophisticated chemical structure of xanthan may be play an important role in the above explanation.

From the above results, it could be concluded that a 1.5 % of xanthan solution could be use as impregnating agent for paper conservation from fungal growth, but these reasoning need a more elaborating studies, when evaluating the results, it would be an advantage if the number of samples had been higher, using different kind of papers and increasing the incubation time. The uncertainty in conclusions and trends increases when the number of samples is low.

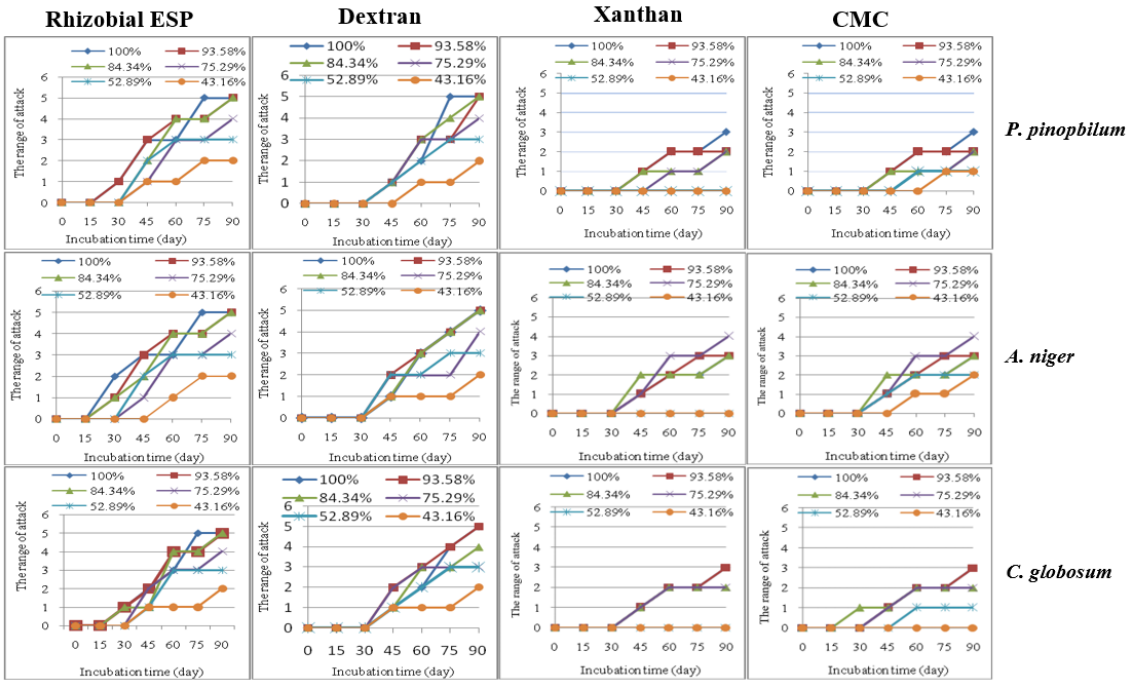


Figure. 6. Growth of different fungi on Whatman paper covered with 1.5 % of microbial biopolymer and CMC during 90 days at 25°C in different relative humidity (the growth represented by range of attack).

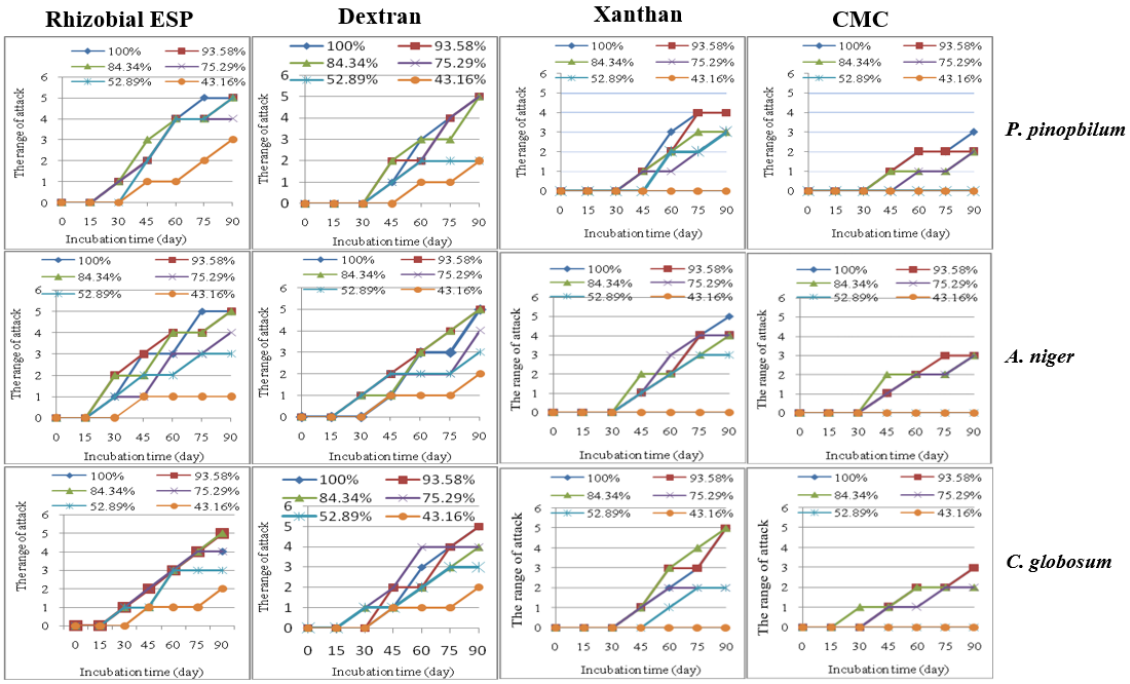


Figure. 7. Growth of different fungi on newspaper treated with 1.5 % of microbial biopolymer and CMC during 90 days at 25°C in different relative humidity (the growth represented by range of attack).

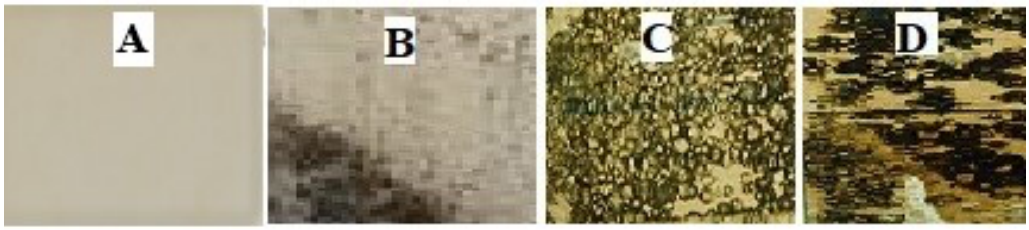


Figure. 8. The growth of *A. niger* on newspaper treated with 1.5 % Rhizobial exopolysaccharides; A: No visible attack, B: Generally abundant, C & D: Very abundant

5. Conclusion

Microbial exopolysaccharides (EPS) played an important role as an ingredient for a variety of industrial applications as natural gums. Three bacterial strains namely Xanthomonas campestris, Rhizobium legumino Sarum and LeuconoStoc mesenteroides were selected as producers of polysaccharides such as dextran, xanthan and Rhizobial exopolysaccharides through cultivation in batch and continuous fermentations in bioreactor. The maximum production of xanthan and dextran were obtained by adding a fresh medium in bioreactor at 182 ml/h (0.091 h-1 dilution rate) and 288 ml/h (0.144 h-1 dilution rate), respectively, while the maximum concentration of Rhizobial exopolysaccharides was recorded at 136 ml/h (0.069 h-1 dilution rate). Continuous cultivation of X. campestris, R. legumin Sarum and L. mesenteroides increased the xanthan, Rhizobial exopolysaccharides and dextran production 23.6, 46.5 and 16.2%, respectively, comparing with bioreactor as a batch culture. Tests were undertaken to evaluate improvements in mechanical properties induced by the microbially produced exopolysaccharides biopolymers on two types of papers Whatman paper and newsprint papers and compared with eight commercial polymers used broadly in paper consolidation. It was found that the exopolysaccharides of xanthan tested acted as reinforcement agents when introduced in the paper, significantly increasing the mechanical properties in comparison with the commercially available polymers used in paper conservation. The effect was significant in different papers tested which demonstrated the protection and strengthening of brittle paper. In addition, to overcome the fungal growth on the treated papers, a 1.5% concentration of Xanthan functioned as mold inhibitors; no visible fungal growth was recorded for 90 days in low humidity on Whatman paper treated with Xanthan.

Conflicts of interest

"There are no conflicts to declare".

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