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Change in bacterial community and pollution load in lab-scale bioreactors designed with waste material for the treatment of paper and pulp mill effluent

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Abstract

Two lab-scale airlift bioreactors using different waste material such as coconut shells (ALBRc) and gravel (ALBRg) as substrates for bacterial growth and a rotating biological contactor (RBC), were designed for the treatment of paper mill effluents. Change in community during the treatment was assessed and it was found that biodiversity of the bacterial species changed during the acclimatization process, with the replacement of some species with the dominant ones at the end of acclimatization. The results showed Bacillus sp., Flavobacterium sp. Pseudomonas sp. and Arthrobacter sp. as the most dominant species. Maximum bacterial growth was observed in ALBRc (120×10^6 CFU/ml), followed by RBC (98×10^6 CFU/ml). Hence, ALBRc was the most effective bioreactor for the microbial biomass production and also for wastewater treatment of paper mill effluents, maximum reduction in pollution load (pH, EC, TDS, BOD, COD and phosphates) was observed in case of ALBRc, (7.05%, 32.9%, 33.9%, 41.8%, 53.7% and 37.4%, respectively). Therefore, it can be concluded that the bacteria related to nutrient removal dominate in the bioreactors and at the same time make the system efficient not only for the wastewater treatment but for biomass production as well.

Keywords: Lab scale bioreactors, waste material, bacterial community, acclimatization, paper and pulp mill effluent.

Introduction

Worldwide, increasing industrialization trend has resulted in the release of industrial effluents at extreme levels into the environment. Paper and pulp sector is one among the most resource demanding (such as raw material, chemicals, energy and water) and pollution generating sector but largely employs conventional technologies.

Central Pollution Control Board (CPCB) has also identified paper and pulp mills among the 17 most polluting industries. Effluent released from pulp and paper industries into the natural water bodies not only adversely affects the water quality but the aquatic life also. Some compounds in the paper and pulp mill effluents are highly recalcitrant and tend to bioaccumulate in the aquatic food chain[1]. Therefore, polluting potential of pulp and paper mill effluent cannot be ignored and requires efficient and economical treatment. Over the years, attempts have been made to to treat the pulp and paper mill effluents using different conventional methods. However, even the most effective conventional methods have severe drawbacks such as high installation cost, operational cost, working unreliability and generation of secondary pollutants[2].

Biological approaches often possess advantages over the conventional ones due to rapid biodegradation rates, low sludge yield and high process stability. Among the various biological methods, bioreactors are most effective for biological elimination of recalcitrant compounds. A bioreactor may contain mass of competent microorganisms immobilized in the form of biofilms. The design of a bioreactor may be with aerobic, anaerobic and micro-aerobic conditions. In aerobic process, the method of aeration has resulted in mechanical agitation reactors, air lift columns, bubbler column and membrane reactors[3].

The application of biofilm reactors for wastewater treatment has many advantages including efficient microbial biomass, high efficiency, small reactor space and lower sludge production[4]. Bacteria in aquatic environments are tend to attach to the surfaces and form a biofilm, which is a multi-species community[5,6].

The structure of biofilms is strictly dependent on the different habitats and environmental conditions of bioreactor[7]. Therefore, the present study was designed with the objectives to identify and evaluate the changes in microbial communities (bacterial) and reduction in pollution load during pulp and paper mill effluent treatment by three different lab - scale biological reactors viz., two airlift bioreactors (ALBR), using waste material such as coconut shells and gravel as substrate for bacterial growth and a rotating biological contactor (RBC) using discarded compact discs of computers as substrate for bacterial growth.

Material and methods

Chemicals and Media: All the chemicals and media used in the present investigation were of analytical grade and procured from standard manufacturer HiMedia Laboratories Ltd., Mumbai, and Merck India Ltd., Mumbai. All the glassware was supplied by Borosil, India.

Sample collection: Sample was collected from Saisons Paper Industry village Bhakli, Pehowa Kurukshetra Haryana.

Bioreactor design: The aim of this study was to utilize waste material for treatment of waste water. Three lab scale bioreactors viz., two airlift bioreactor (ALBR) using different substrates and a rotating biological contactor (RBC) using waste computer discs as substrate for biofilm formation were constructed for the treatment of effluent (Figure-1). For ALBR, two used Bisleri plastic bottles of 20l volume were taken and connected in parallel with the air pump. Container 1 contained coconut shells (named as ALBRc) and container 2 contained gravel (named as ALBRg) as substrate for microbial growth. Each container was filled with 14l of paper mill effluent supplemented with minimal salt media (MSM). Two air pumps were used for aeration of containers separately for proper mixing of nutrients and proper growth of microorganisms.

For RBC lab scale reactor discarded compact disks (CD) of computer (12cm diameter) were used as substrate for biofilm formation. For RBC reactor a circular plastic tub was used for storage of effluent. In this tub the effluent (14L) was filled (upto a height of 7cm) and just above 2cm from effluent level, two holes were provided for fixing two horizontal shafts at a distance of 5cm. The CDs which were used as a disk contactor were first roughed with abrasive to make the surface rough and increase surface area for bacterial growth. These CDs were placed in each shaft at a distance of 2cm to contact the whole volume of effluent of plastic tub. In the above RBC bioreactor almost 40% of the CD disk was allowed to submerge in wastewater and shaft was rotated at speed of 6 rpm for providing aeration. For rotation of shafts an electric motor was used. The surface area for biomass growth was 7702.8cm^2 considering both side of 15CDs. These discarded disks were made up of plastic and were easily available. The corrugated plastic pipe was used to fix the discs on shafts so that the disc could easily rotate with the shaft. It was anticipated that maximum aerobic bacterial growth will occur on disk due to more rough surface area as compared to smooth surface.

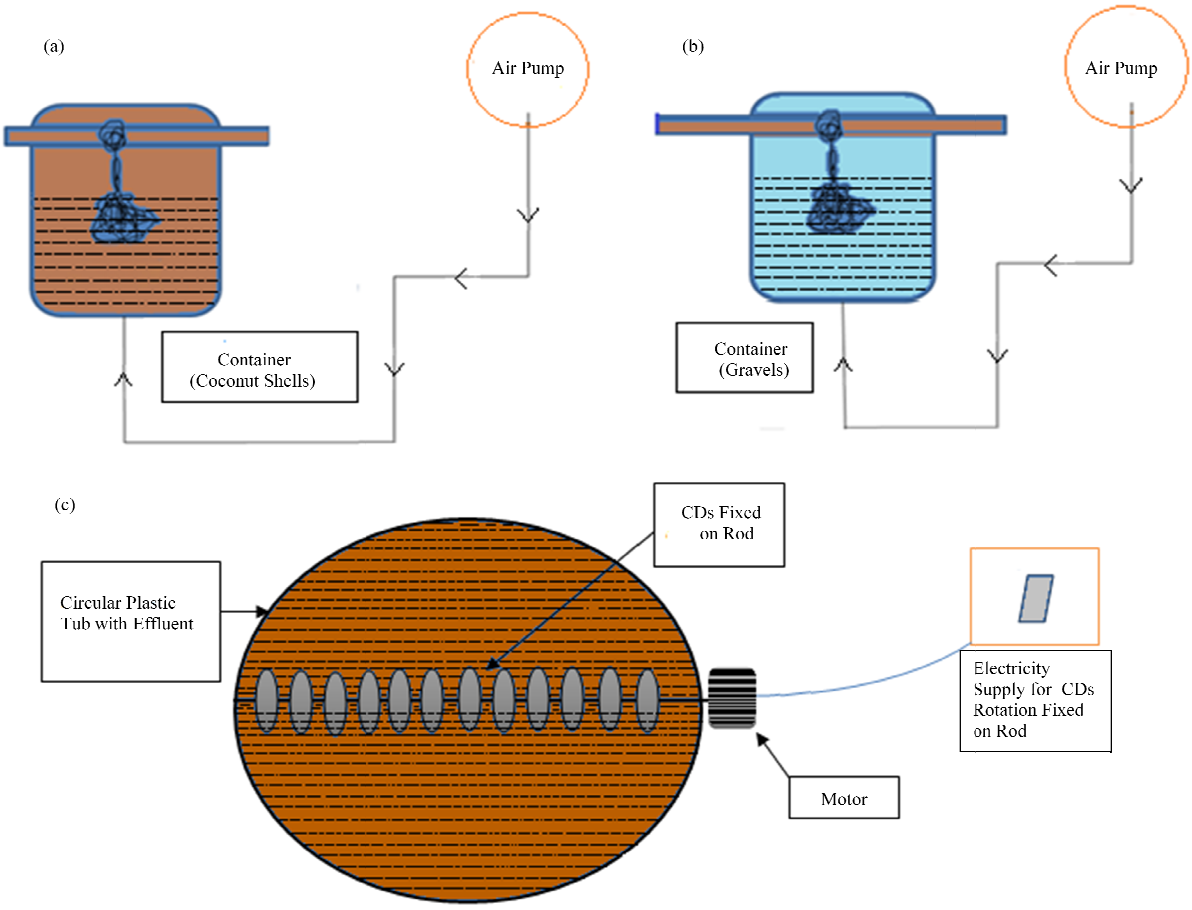

Figure-1: Layout of Bioreactors (a) ALBRc (b) ALBRg (c) RBC.

Acclimatization of bioreactor: Acclimatization is a process of adaptation of microbial species to new environmental conditions over time. All the bioreactors were acclimatized for 2 weeks under same environmental conditions prior to effluent treatment during which the bacterial populations were supposed to show active growth which leads the biofilm formation around the different substrates (coconut shell, gravel and discs) provided for growth. Each reactor was then filled with paper industry effluent (14l).

The effluent was supplemented with MSM to stimulate the growth of bacteria. Effluent samples were collected on 6^{th} and 12^{th} day to study bacterial activities. For this, biofilm was scraped from different substrates and mixed in sterilized distilled water by gentle shaking. Bacterial colonies were isolated on nutrient agar media (NAM) by serial dilution methodology (10^{-1} to 10^{-6}). Further the pure cultures were obtained on NAM plates by streaking method.

Identification of most effective bacterial isolates involved in treatment of paper mill: After the acclimatization period of 2 weeks, it was supposed that the bacterial growth has lead to sufficient biofilm cover around the coconut shell, gravel bed and disk surface. Microbial characterization was further done morphologically and biochemically to identify most effective strains involved in the treatment of effluent[8].

Statistical analysis: All the experiments were done in replicates of three; data was statistically analyzed in Microsoft Excel 2007. Values are presented as mean of three replicates with standard error.

Results and discussion

Microbial characterization of sludge used for inoculation: Microbial community analysis of sludge from paper and pulp mill was done prior to its use for the inoculation in three different bioreactors (ALBRc, ALBRg, RBC). The operation of bioreactors relies on the performance of the microbial population present in the sludge i.e. mainly bacteria being responsible for the removal of pollution loads. As they derive their growth requirement from the organic and inorganic pollutants present in effluent and synthesize enzymes and metabolic products such as, structural proteins, lipids and nucleic acids[9]. It is therefore important to characterize different microbial population types present in sludge. Hence, before inoculation of sludge in bioreactors (ALBRc, ALBRg, RBC), its microbial characterization was done by morphological, microscopic and biochemical analysis of isolated strains.

Quantitative estimates of the microbial population observed on NAM plates were done by CFU count. A total of six different colonies (S1–S6) were obtained with total CFU count of 80×10^6 per gram dry weight of sludge, of which S1 was recorded to be dominating with 22×10^6 CFU/g followed by S3 (14×10^6CFU/g), and the least being S5 (9×10^6CFU/g). Table 1 shows morphological, microscopic (gram staining) and biochemical characteristics of the isolates. All the strains appeared to form white colonies with smooth surface. The cell morphology was studied by examining the stained bacterial culture under microscope (at 100X).

The strains S1, S2 and S4 were observed as gram positive with cell shapes cocci, rod and cocci respectively and arranged as tetrads (S1) and chains (S2 and S4), while the other strains S3, S5 and S6 were gram negative, small rods arranged in chain and/or single. All the isolates were further studied for biochemical tests such as starch hydrolysis, casein hydrolysis, gelatin hydrolysis and carbohydrate utilization test by inoculating each strain in different media available for specific biochemical tests (Table-1).

Among different isolates S1 showed positive test for gelatin hydrolysis and carbohydrate utilization, S2 for starch hydrolysis, S3 for starch and gelatin hydrolysis, S5 for gelatin hydrolysis and carbohydrate utilization and S6 for starch, casein and gelatin hydrolysis. The positive results obtained for starch, gelatin hydrolysis, casein and carbohydrate test shows that bacterial isolates possess alpha-amylase, gelatinase enzyme, caseins hydrolysis and carbohydrate utilization activities respectively. On the basis of colony morphology, microscopic and biochemical properties, strains S1, S2, S3, S5 and S6 were identified as *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Escherichia coli* and *Alcaligen* sp. respectively. Biochemical properties of these isolates have also been reported by several workers[9-12]. Among all the isolates strain S4 showed negative response to all biochemical test, therefore could not be identified and was considered as unknown.

Change in the bacterial community in three bioreactors during acclimatization: Microbial community characterization during the different acclimatization period of a bioreactor is determined as it favors their appropriate operation. Bacterial community developed during the 6^{th} and 12^{th} day of acclimatization was studied and the characterization was done by morphological and biochemical test. During this study, the obtained bacterial profile was highly different from that obtained in sludge. Sipma et al.[13], stated that an overall effective performance of a wastewater treatment system can be achieved with microbial communities, which are not only able to perform under normal operating conditions but also can adapt to changes in the wastewater quality during the continuous treatment process. Six different bacterial colonies with varied population density were observed in biofilm formed on different substrate of all bioreactors at 6^{th} day of acclimatization. Among the six colonies, PS4 was recorded as predominate with 39×10^6 CFU/ml and least density was recorded for PS5 (15×10^6 CFU/ml). The bioreactor ALBRc supported maximum bacterial growth with total viable count of 159×10^6CFU/ml (Figure-2). This may be due to availability of large amount of rough surface on coconut shell for the biofilm formation as compared to other substrates such as gravel and discs used in ALBRg and RBC, respectively[14].

Table-1: Morphological characterization of bacteria isolated from sludge used for inoculation in all bioreactors.

Characteristics	Bacterial Isolates					
	S1	S2	S3	S4	S5	S6
Size	Medium	Large	Medium	Medium	Medium	Medium
Color	White	White	White	White	White	White
Colony elevation	Raised	Convex	Flat	Raised	Convex	Convex
Colony margin	Entire	Irregular	Entire	Entire	Entire	Entire
Gram staining	+	+	-	+	-	-
Cell shape	Cocci	Rod	Small rod	Cocci	Small rod	Small rod
Cell arrangement	Tetrad	Small Chain	Chain	Chain	Single	Single
Biochemical tests						
Starch Hydrolysis	-	+	+	-	-	+
Casein Hydrolysis	-	-	-	-	-	+
Gelatin Hydrolysis	+	-	+	-	+	+
Carbohydrate Test	+	-	-	-	+	-

'-' negative; '+' positive.

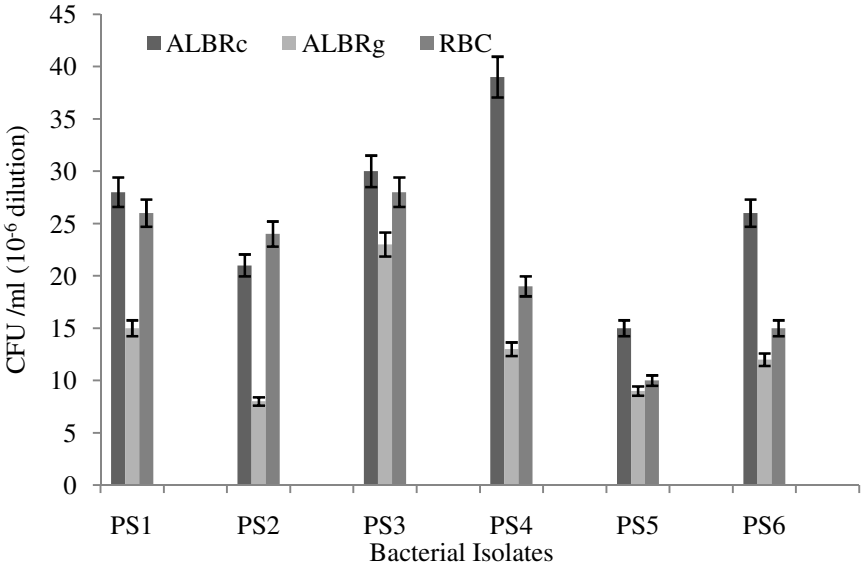

Figure-2: Bacterial count in biofilm samples of three bioreactors ALBRc, ALBRg and RBC after 6 days of acclimatization.

Bacterial population count in wastewater of different bioreactors revealed that the reactor containing coconut (ALBRc) as substratum for biofilm formation significantly immobilized the bacterial population and reduced the release of cells into the wastewater as compared to the disks and stone containing bioreactors. Among the six isolates, only two (PS1 and PS 6) were observed suspended in the wastewater of ALBRc with a total viable count 9×10^6CFU/ml and 6×10^6CFU/ml, respectively, whereas, in ALBRg wastewater three isolates PS1, PS5 and PS6 were observed to be suspended (Table-2). After quantitative estimation cultures were further examined for morphological and biochemical properties (Table-3). The

comparative study of results with Bergey's manual showed that all isolates except PS3 were gram positive. Most strains produced pigments on nutrient agar plates, with yellow and orange as predominant. The microscopic observations revealed the presence of rod shaped cells arranged in chain as predominant, except PS5 that appeared as cocci shaped cells. Among the biochemical tests, positive starch test was given by PS1, PS2, PS3, PS4 and PS6; casein test by non of the isolates, gelatin test by PS2 and PS6 only and carbohydrate test by all isolates (Table-3).

Table-2: Total count of bacterial isolates observed as suspended in the wastewater of three different bioreactors after 6 days of acclimatization.

Bacterial Isolates	Bioreactors		
	ALBRc	ALBRg	RBC
PS1	ND	ND	ND
PS2	9×10^6	13×10^6	9×10^6
PS3	ND	ND	ND
PS4	ND	ND	ND
PS5	ND	5×10^6	ND
PS6	6×10^6	14×10^6	10×10^6

ND = Not Detected.

Table-3: Morphological and biochemical characterizations of bacteria isolated from ALBRc, ALBRg and RBC reactors after 6 days of acclimatization.

Characteristics	Bacterial Isolates					
	PS1	PS2	PS3	PS4	PS5	PS6
Size	Small	Large	Small	Small	Small	Medium
Color	Yellow	White	White	Peach	Yellow	White
Colony Elevation	Convex	Flat	Convex	Convex	Convex	Raised
Colony Margin	Entire	Entire	Entire	Entire	Entire	Entire
Gram Staining	+	+	-	+	+	+
Cell Shape	Rod	Rod	Small Rod	Small Rod	Cocci	Rod
Cell Arrangement	Chain	Long Chain	Single	Chain	Chain	Single
Biochemical tests						
Starch Hydrolysis	+	+	+	+	-	+
Casein Hydrolysis	-	-	-	-	-	-
Gelatin Hydrolysis	-	+	-	-	-	+
Carbohydrate Test	+	+	+	+	+	+

'-' negative; '+' positive.

On the basis of morphological and biochemical results, isolates PS1, PS2, PS3, PS4, PS5 and PS6 were identified as *Flavobacterium sp., Bacillus sp., Pseudomonas sp., Brevibacterium sp., Arthrobacter sp., Bacillus sp.* respectively. It was observed that isolates PS2 and PS6 showed similar properties for the utilization of different carbon sources but differ in cell morphologies, thus these were considered as two different species belonging to genus *Bacillus*. In the present study, *Brevibacterium* sp. and *Flavobacterium* sp. were observed to be dominating and most effective in utilizing the organic content of wastewater. Sharifi-Yazdi et al.[9] also identified genus of *Brevibacterium* sp., *Flavobacterium* sp., and *Pseudomonas* sp. during the treatment of industrial effluent.

On 12th of acclimatization study, a high variability between the bacterial populations of inoculums (sludge) and that of the bioreactors were observed, although some strains were common. The bioreactor operation conditions such as aeration mode, substrate use for biofilm formation etc significantly induced the endogenous microbial community structure during the acclimatization[15]. Of bacterial community developed during the 12th day, some bacterial isolates (PS1, PS2 and PS5) were identical to the 6th day of acclimatization study. Eight different bacterial colonies with varied population density were observed in biofilm formed on different substrate of all bioreactors. Among the eight, PS7 was recorded as predominant with 24×10^{6}CFU/ml in ALBRc reactor (Figure-3).

The bioreactor ALBRc supported maximum bacterial growth (total viable count of 129×10^{6}CFU/ml) that may be due to availability of large amount of rough surface on coconut shell for the biofilm formation as compared to other substrates such as gravel and discs used in ALBRg and RBC, respectively. Bacterial population count in wastewater of different bioreactors revealed that the reactor containing coconut (ALBRc) as substratum for biofilm formation significantly immobilized the bacterial population and reduced the release of cells into the wastewater as compared to the disks and gravel containing bioreactors. Among the eight isolates only three (PS7, PS2 and PS5) were observed suspended in the wastewater of ALBRc and ALBRg with different total viable count (Table-4).

After quantitative estimation, cultures were further examined for morphological and biochemical properties. The results showed that isolates PS7, PS10 and PS11 were gram positive and PS 9 was gram negative (Table-5). The microscopic observations revealed that gram positive rod cells arranged in chain predominated, except PS5, PS10 and PS11 that appeared as coccus cells. Further the isolates were subjected to biochemical tests and the results reveal that among all the isolates, PS1, PS8 and PS2 showed positive starch test, whereas PS7, PS9, PS10, and PS11 isolates showed negative starch test; positive casein test was observed in PS7 and PS9 isolates only, positive gelatin test in PS8, PS2, PS10 and PS11 isolates and positive carbohydrates test in PS1, PS2, PS5 and PS11 (Table-5). Isolate PS2 gave maximum positive results for number of biochemical tests suggesting that the isolate is highly efficient towards the utilization of variety of carbon sources and thus makes it suitable of its application in treatment of wastewater. On the basis on morphological and biochemical results, isolates PS7, PS8, PS9, PS10 and PS11 were identified as *Bacillus* sp., *Pseudomonas* sp., *Comamonas* sp., *Staphylococcus* sp. and *Enterococcus* sp. respectively.

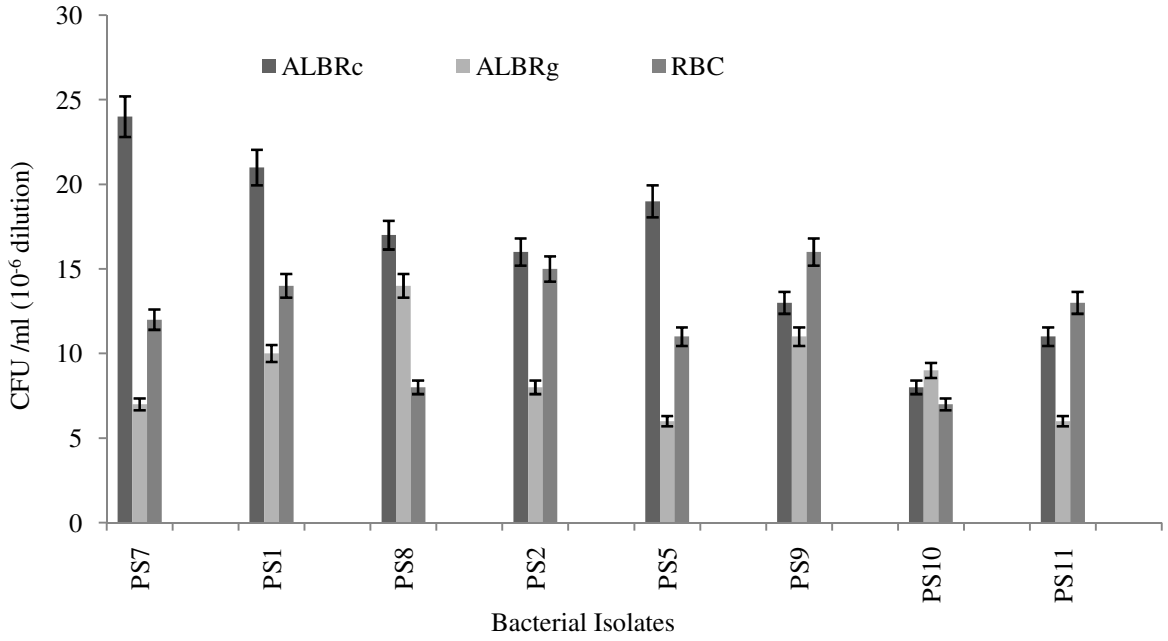

Figure-3: Bacterial count in biofilm samples of three bioreactors ALBRc, ALBRg and RBC after 12 days of acclimatization.

Table-4: Total counts of bacterial isolates observed as suspended in the wastewater of three different bioreactors after 12 days of acclimatization.

Bacterial Isolates	Bioreactors		
	ALBRc	ALBRg	RBC
PS7	11×10^6	12×10^6	11×10^6
PS1	ND	ND	ND
PS8	ND	ND	ND
PS2	8×10^6	6×10^6	ND
PS5	9×10^6	13×10^6	10×10^6
PS9	ND	ND	ND
PS10	ND	ND	ND
PS11	ND	ND	ND

ND = Not Detected.

Table-5: Morphological characterizations of bacterial isolates observed in ALBRc, ALBRg and RBC reactors after 12 days of acclimatization.

Characteristics	Bacterial Isolates							
	PS7	PS1	PS8	PS2	PS5	PS9	PS10	PS11
Size	Medium	Small	Small	Large	Small	Very Small	Small	Small
Color	White	Yellow	White	White	Yellow	White	Peach	Yellow
Colony elevation	Convex	Convex	Convex	Flat	Convex	Convex	Convex	Convex
Colony margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Gram staining	+	+	-	+	+	-	+	+
Cell shape	Long Rod	Rod	Small Rod	Rod	Cocci	Rod	Cocci	Cocci
Cell arrangement	Chain	Chain	Single	Long Chain	Chain	Single	Cluster	Chain
Biochemical tests								
Starch hydrolysis	-	+	+	+	-	-	-	-
Casein hydrolysis	+	-	-	-	-	+	-	-
Gelatin hydrolysis	-	-	+	+	-	-	+	+
Carbohydrate test	-	+	-	+	+	-	-	+

'-' negative; '+' positive

Identification of most effective bacterial isolates involved in the treatment of paper mill effluent: After acclimatization period all the bioreactor were run for the treatment of effluent for 8 hrs. At the end of the treatment, biofilm samples were collected from different substrates used in ALBRc, ALBRg and RBC for characterization of bacterial profile involved in treatment process. The results obtained during this study were identical to the bacterial profile recorded at 12th day of acclimatization period. This may be due to bacterial population of bioreactor that may have stabilized at 12th day of acclimatization. Total eight types of colonies were observed in bioreactors with varied number of total count (Table 6). In ALBRc seven isolates were observed with total count 120×10^6 CFU/ml as compared to ALBRg (eight isolates with total count 76×10^{6}CFU/ml) and RBC 98×10^{6}CFU/ml. This higher bacterial population growth shows that ALBRc has favorable biomass growth on coconut shells as compared to RBC and ALBRg. The results reveal that *Bacillus* sp., *Flavobacterium* sp. *Pseudomonas* sp. and *Arthrobacter* sp. are the most dominant and therefore efficient among all the isolates for the biodegradation of pollutants present in the pulp and paper mill effluent. *Staphylococcus* sp. and *Enterococcus* sp. were less in number, thus least efficient for the biodegradation of wastewater among the 8 isolates.

Change in the pollution parameters of effluent in the bioreactors after the treatment: The physicochemical properties of effluent generated from pulp and paper mill are given in Table-7. The effluent used in the present investigation showed following characteristics i.e. pH 8.5, EC 4.10mS/cm, TDS 1348mg/L, BOD 1255mg/L, COD 2482mg/L and phosphates 28mg/L (Table-7). During the pulping process, use of high amount of sodium hydroxide and sodium sulfite contributes the alkalinity to the effluent, which is therefore cause rise in pH levels of wastewater[16]. Various organic and inorganic compounds and lignin derivatives released during the bleaching of pulp contributes high levels of BOD and COD to the effluent[17]. In all the lab scale bioreactors the results showed marked decline after the treatment of 8 hrs. A reduction of 7.05%, 32.9%, 33.9%, 41.8%, 53.7% and 37.4% in pH, EC, TDS, BOD, COD and phosphates, respectively was observed in ALBRc treatment bioreactor (Table-7). In ALBRg bioreactor different pollution parameters such as pH, EC, TDS, BOD, COD and phosphate of effluent were reduced by 12.9%, 32.9%, 62%, 54.8%, 56% and 43% respectively, after 8 hrs treatment. Whereas, in RBC a reduction of 7.05%, 34%, 34%, 50.7%, 67% and 42% of pH, EC, TDS, BOD, COD and phosphates, respectively, of effluent was recorded after 8 hrs of treatment. Therefore, the results of present study showed that maximum reduction in pollution of paper and pulp mill effluent was observed in ALBRg followed by ALBRc and RBC. These results of the present study could be explained assuming that reduction in pollution load after treatment most probably occurred due to degradation of organic pollutants and other colloidal particulates in the effluent by the microbial activities at higher rate in ALBRg compared to ALBRc and RBC.

Table-6: Bacterial count in biofilm samples of three bioreactors ALBRc, ALBRg and RBC after wastewater treatment.

Isolates	Counting CFU(Colony Forming Unit)		
	ALBRc	ALBRg	RBC
Bacillus sp.	29×10^6	10×10^6	16×10^6
Flavobacterium sp.	25×10^6	14×10^6	19×10^6
Pseudomonas sp.	18×10^6	12×10^6	10×10^6
Bacillus sp.	15×10^6	11×10^6	20×10^6
Arthrobacter sp.	16×10^6	9×10^6	18×10^6
Comamonas sp.	13×10^6	1 1×10^6	15×10^6
Staphylococcus sp.	4×10^6	8×10^6	ND
Enterococcus sp.	ND	1×10^6	ND
Total CFU Count	120×10^6	76×10^6	98×10^6

ND – Not Detected.

Table-7: Physico-chemical characteristics of black liquor before and after treatments of 8 h in different lab scale bioreactors (ALBRc, ALBRg and RBC).

Parameters	Effluent Value Before Treatment	After Treatment		
		ALBRc	ALBRg	RBC
pH	8.5 ± 0.03	7.9 ± 0.09	7.4 ± 0.04	7.9 ± 0.03
EC (mS/cm)	4.10 ± 0.06	2.75 ± 0.05	2.75 ± 0.08	2.7 ± 0.09
TDS	1348 ± 1.66	890 ± 1.23	509 ± 1.09	886.6 ± 1.13
BOD	1255 ± 1.21	730.4 ± 1.46	567.26 ± 2.11	618 ± 1.90
COD	2482 ± 3.11	1149.17 ± 2.06	1092.08 ± 1.23	814 ± 4.03
Phosphates	28 ± 0.93	17.53 ± 1.01	15.96 ± 1.31	16.24 ± 0.99

Note: Values are given as mean of three replicates ± S.E.

Conclusion

The results of the present investigation on assessment of bacterial community in three different lab scale bioreactors, designed with waste material used for the treatment of pulp and paper mill effluent showed that different types of bacterial community were involved in different bioreactors (ALBRc, ALBRg and RBC). It was clear that the bacterial populations observed in sludge used for inoculation changed with time and became stable after the period of acclimatization. At the same time, bacteria related to nutrient removal might have been enriched and become stable as observed along the successive tests. Some bacteria in the acclimatized bacterial diversity were not present in the inoculums, suggesting that some new communities were enriched after operation. During this study, the obtained bacterial profile was highly different from that of the first obtained in sludge. The results showed that the bioreactor ALBRc supported maximum bacterial growth followed by RBC and ALBRg. Bacterial population count in wastewater of different bioreactors revealed that the reactor containing coconut (ALBRc) as substratum for biofilm formation significantly immobilized the bacterial population and reduced the release of cells into the wastewater as compared to the disks and stone containing bioreactors. It is clear from the study that among all the three bioreactors ALBRc is the most effective bioreactor followed by RBC and ALBRg for the biomass production that can be utilized for the production of protein cells and enzymes of industrial application. However, ALBRg showed best application in terms of reduction in pollution load for the purpose of wastewater treatment. Thus, such lab scale bioreactors indicate the potential of utilization of waste material, biomass production and paper and pulp mill effluent treatment.

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