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Bacterial amelioration of bauxite residue waste of industrial alumina plants

MK Hamdy[1] and FS Williams[2]

[1]*Food Science and Technology, University of Georgia, Athens, GA 30602, USA;* [2]*Alcoa World Alumina Atlantic, Point Comfort, TX, USA*

The high alkali content of bauxite residue deposits from alumina production plants in industrial nations poses a challenge to reestablish flora and fauna at the deposit sites. The present study demonstrated that low levels of injured bacterial cells in the bauxite residue actively grew using various added nutrients and/or hay. The organisms grew from less than 10 to more than 10^9 cells g^{-1} bauxite residue and formed organic acids that lowered the pH from 13 to about 7.0. A total of 150 cultures was isolated from treated bauxite residue and included species of *Bacillus*, *Lactobacillus*, *Leuconostoc*, *Micrococcus*, *Staphylococcus*, *Pseudomonas*, *Flavobacterium* and *Enterobacter*. Scanning electron micrographs demonstrated that untreated particles (control) of the bauxite residue were clumped together, and in treated bauxite residue these particles were highly dispersed with microcolonial structures. Furthermore, the treated bauxite residue supported growth of several plants and earthworms that survived for over 300 days. In a test plot bioremediation on a residue deposit at Alcoa Point Comfort, TX, the Bermuda grass hay used was effective mulch material and encouraged water filtration, leading to establishment and growth of salt-tolerant vegetative species. *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 228–233.

Keywords: bauxite residue waste; bacterial amelioration; scanning electron micrographs

Introduction

Bauxite residue is formed during heat treatment of bauxite ores in sodium hydroxide solution using the Bayer process leading to soluble alumina as $Al(OH)_3$. The remaining insoluble materials (principally iron oxide) are known as red mud or bauxite residue [12]. The magnitude (tons per year) and the chemical analyses of the bauxite residues produced by three major industrial alumina plants in USA are recorded in Table 1. The chemical and mineralogical composition of this residue, including its particle size distributions, are variable due to grade differences in bauxite ore and the process operating conditions. The bauxite residue is a highly alkaline mixture of fine particle-sized metal oxides and the most common method for its disposal is storage in impounded, diked deposits adjacent to the aluminum processing plants. The free moisture content of the bauxite residue is variable (30% to 60%), depending on the disposal method, and consists of dilute caustic solution with a pH of about 13. Keeping the residue behind constructed dikes is not the ideal storage since bauxite residue lakes require a large land space and the soil properties of the final deposit may make it impossible to support heavy equipment or construction at the site [12]. The contained deposits also pose a potential risk of groundwater contamination hinging on the hydrogeology of the site.

Attempts have been made to recover minerals present in bauxite residue such as iron for the steel industry, or to utilize the residue as a raw material for road building. However, bauxite does not offer unique or beneficial properties as a raw material for these applications. The potential use of bauxite residue for recovery of aluminum by chemical or biological leaching (*Penicillium simplicissimum*) was reported [14], and Edwards *et al* [8] suggested using bauxite residue as a lime substitute for treating acid soils as well as acid mine drainage sites and to enhance mineral-deficient soils. Anand *et al* [2] used *Bacillus polymyxa* for the removal of calcium and iron from bauxite ore. Attempts were made to rehabilitate the residue deposits and bauxite mines with and without neutralization, by establishing vegetation cover using indigenous plants that are able to survive the adverse conditions [15]. Gypsum ($CaSO_4$) and copperas ($FeSO_4$) were suggested [16] as ameliorants for red mud; the addition of these acidic wastes at 8% released Na and Ca from red mud and reduced the pH to about 8.5. In the present investigation, probing techniques were developed targeting bacterial treatment of bauxite residue by inducing the metabolically injured bacteria (intrinsic to bauxite residue) to repair, using specific nutrients, and to grow to high levels forming organic acids that neutralized the alkalinity of the bauxite residue converting it to arable land. Factors affecting the bioremediation included aeration, concentrations of the nutrients in the medium including hay, and the ratio of bauxite residue to medium. Changes in the physical structure of the untreated (control) versus the treated bauxite residue were examined using scanning electron microscopy. This bioremediation treatment was also used to revegetate a bauxite residue demonstration plot at Point Comfort, TX with good success.

Materials and methods

Six 10-l samples from Alcoa bauxite residue lake deposit at Mobile, AL were collected in sterile laminated stainless steel containers. The first and second samples were obtained from the bauxite residue lake deposit near the dike and below the residue surface (0–5 and 5–20 cm, respectively), the third and fourth were 20 m away from the dike and also below the surface (1–5 and 5–20 cm, respectively). The fifth sample was fresh slurry, secured directly from the processing plant, and the sixth was lake water from the

Correspondence: Dr MK Hamdy, Food Science and Technology, University of Georgia, Athens, GA 30602, USA
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Table 1 The magnitude of the production (metric tons per year) and the chemical analyses (percent dry weight) of bauxite residue from the three major United States alumina processing companies[a]

Chemicals assayed	Chemical analyses (% dry wt.)		
	Kaiser 800,000[b]	Alcoa 1,560,000[b]	Reynolds 1,510,000[b]
Al_2O_3	15.00	17.8	8.89
Fe_2O_3	51.50	40.0	52.5
SiO_2	1.70	9.59	4.48
TiO_2	6.70	8.48	6.64
CaO	7.00	7.57	10.85
Na_2O	0.97	2.69	3.17
LOI[c]	9.30	10.3	8.46

[a]Locations: Kaiser — Gramercy, LA; Alcoa — Point Comfort, TX; Reynolds — Corpus Christi, TX.
[b]Tons per year.
[c]Loss on ignition at 100°C.

impounded bauxite residue lake. All six samples were examined for pH and microbial contents (aerobic and anaerobic), before and during the bioremediation, using minimal medium, tryptic soy broth and/or agar for the aerobes, and the same medium for anaerobes containing 0.1% fluid thioglycolate plus 0.1% Na-ascorbate [1,3]. All six samples were assayed chemically (Table 2).

Microbial analyses

Samples were assayed for pH and for both viable and injured bacterial cells using minimal and enriched media as well as utilizing the hanging-drop slide method with the methylene blue reduction test and viewed microscopically. The method [9,10] uses the changes in methylene blue from blue in the oxidized state (E_h of +71 mV) to colorless form in the reduced state. Viable bacterial cells use methylene blue as the hydrogen acceptor *via* the dehydrogenases in the presence of NADH and appear colorless whereas dead cells stain dark blue. Metabolically inactive bacterial cells exhibit shades of light blue depending on the level of active dehydrogenases. Furthermore, injured cells grow on enriched media (TSB and/or TSA) but not on minimal basal broth or agar media. The shake culture tube method was used for anaerobes and all results for both viable and injured bacterial counts were an average of three to four experiments reported as colony forming units (CFU) g^{-1} sample.

Repair of metabolically resting bacteria

Several enrichment media and other added nutrients [4,7,10,13] were used to induce repair and growth of the inactive bacteria intrinsic to bauxite residue. These types of nutrients included: ATP, basal (salts) broth, tryptic soy broth, glucose, yeast extract, peptone, fresh and old paper pulp wastes and different types of hay. The nutrients were dissolved in deionized water and utilized in either nonsterile or sterile medium [filtration (0.45 μm) and/or autoclaved at 121°C for 15–20 min]. The desired nutrients, dissolved in water, were mixed with bauxite residue in various ratios and all aerobic samples were kept in a shaker incubator, at a specified temperature, to enhance growth before plating them. Concentrated nutrients (in H_2O) were prepared (10×) and the desired volume was added at intervals as promoters of bacterial growth in the bauxite residue mixture. Fresh and old paper pulp were obtained from Mobile, AL.

Hay analyses

Bales of Bermuda grass and/or alfalfa, obtained from a local market, were used and each was ground in a Wiley mill to fine powder (1-mm mesh), placed in sterile glass jars and stored at 4°C. Assays of total reducing sugars and total carbohydrates present in the hay extracts were assayed. Identification of sugar was conducted using both paper and thin-layer chromatography [5] and compared to standards. Spots on the chromatograms were identified by specific color reactions and the concentration was determined after comparison with known sugar standards. The organic acids present in treated and untreated bauxite residue were separated using two-dimensional high-performance thin-layer chromatography on silica gel G plates [5] and the solvent systems: [ethanol:water:ammonia (100:12:16) and benzene:methanol:acetic acid (90:16:8)]. Chromatographic plates were sprayed with bromphenol blue (0.04% in 95% ethanol), the spots were eluted, and their concentration was compared to standards [5,6].

Bioremediation of bauxite residue in columns

Two glass columns with various ports, located at different distances for withdrawing samples, were used to ascertain the

Table 2 Chemical analyses of the six bauxite residue samples obtained from the plant at the lake (abandoned impoundment at Mobile, AL). Average results were reported as percent dry weight for all samples (1–5) and as grams per liter for the lake water sample (No. 6)

Chemicals assayed	% Weight in sample number[a]					
	1 0–5 cm	2 5–20 cm	3 0–5 cm	4 5–20 cm	5 slurry	6 water
Al_2O_3[b]	18	15	19	16	29	8.5[b]
Fe_2O_3	33	31	23	28	17	15.7 (T.C.)[c]
SiO_2	7	7	10	8	19	8.9 (Na_2CO_3)
Na_2O	2	4	6	5	10	
TiO_2	8	12	12	12	3	
% Solid	71	56	67	31	7	

[a]Sample Nos. 1 and 2 were obtained near the dike, Nos. 3 and 4 about 20 m from the dike, No. 5 was fresh bauxite residue slurry directly from the processing plant and No. 6 was lake water from the impoundment.
[b]Al_2O_3.
[c]Total carbon.

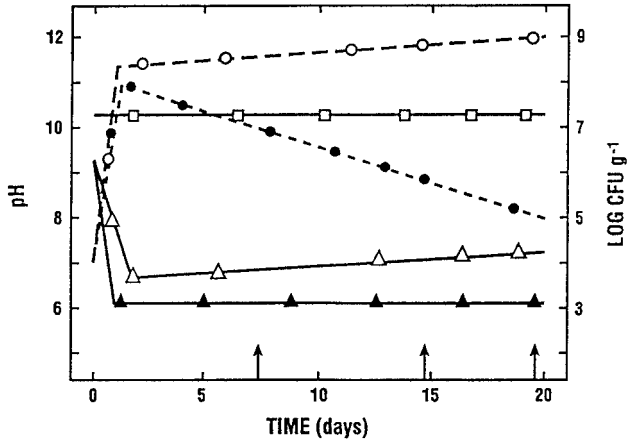

Figure 1 Effect of medium (2:1, w/v) on growth of aerobic (○) and anaerobic (●) bacteria and on the pH of bauxite residue (△ aerobic, ▲ anaerobic, □ control). The bauxite residue used consisted of equal weights from sample Nos. 1–4 and the control sample had water added. The composition of the medium used is listed in the text, and 3.0 ml promoters were added as indicated by the arrows

movement of bacteria and nutrients in the medium through the bauxite residue column. Each column was 91×6 cm I.D. with 12 ports (closed using rubber stoppers) located on both sides. One column had an equal mixture of sample Nos. 1–4 and the medium was placed on top of bauxite residue at a ratio of 2:1 (550 g bauxite residue and 275 ml medium). The medium contained (g l^{-1} water): glucose 30, peptone 2.5, yeast extract 1.5, K_2HPO_4 2 and $CaCO_3$ 0.1. The second column had the same bauxite residue and medium, but both were mixed well before adding them to the column. The columns were kept at a constant temperature of 25°C by circulating water, through the jacket, from a water bath. Samples were withdrawn at intervals from the desired port and assayed for pH and microbial activity.

Survival of plants and animals

Small plants (liriope, pampas grass, cactus, white pine, poplar sapling) and earthworms (red wigglers, night crawlers) were used for both the survival and growth studies in the treated bauxite residue (34 days old, pH 7.6) and in the untreated bauxite residue (control). Each plant or animal was transferred to several earthenware pots containing the bauxite residue sample and observed for growth and survival time.

Scanning electron microscopy

Samples of each bauxite residue (untreated and treated) were fixed with OsO_4 vapor for 24 h, dehydrated by successive ethanol solutions (50, 70, 95 and 100%, v/v) for 15 min and then dried using CO_2 critical point drying [3,11]. Samples were then viewed at 20 kV using a Philips 505 SEM.

Results and discussion

Microbial activities

All bauxite residue and lake-water samples (controls) contained small numbers of metabolically inactive (injured) bacterial cells that grew in enriched, but not in minimal basal medium, and exhibited the light blue color of methylene blue in the hanging-drop slide. These cells grew after incubation at 37°C in the desired medium followed by plating them on tryptic soy agar for aerobes and anaerobes. The number of such cells ranged from an average of 10 to 450 CFU g^{-1} sample. Sterile medium used for the bioremediation had (g l^{-1} water): 0.01 ATP, 20 glucose, 1 yeast extract, and 10 KH_2PO_4 and was used with each bauxite residue sample at a ratio of 5:1 (bauxite residue to medium, w/v). Both aerobes and anaerobes grew well within 1 day in all treated samples to a total of 8×10^7 CFU g^{-1} sample to 10^9 after 20 days for the aerobes, and 9×10^5 CFU g^{-1} for the anaerobes. The pH of treated bauxite residue decreased from an initial value of about 11.0 to 6.5 for all samples after 20 days incubation (37°C) under aerobic or anaerobic conditions. The control sample (bauxite residue in water) showed no changes. The sources of these microbial cells were not determined, but it is possible that they were from the air column above the lake, the dust particles near the dikes, human shedding during sampling at the sites, and/or during transit.

Factors affecting bioremediation

The results from tests, where the ratio of bauxite residue to medium was reduced to 2:1 and nutrients were added to the medium, showed significant growth of aerobes and anaerobes (Figure 1). The bauxite residue used was a mixture of equal weight from sample Nos. 1–4 and the nutrients were (g l^{-1} water): ATP 2, glucose 30, peptone 5, yeast extract 2, KH_2PO_4 2, and $CaCO_3$ 0.1. Within 1 day, growth of aerobes and anaerobes reached counts of 7×10^8 and 8×10^8, respectively, and after 20 days the values were 10^9 and 9×10^5 CFU g^{-1}, respectively. The increased level of microbial activity was due to the nutrients in medium and to the addition of promoter (at intervals), as shown by the arrows. The control (bauxite residue sample No. 2 and water) showed no bacterial growth, as they were metabolically inactive (i.e., grew on enriched medium but not on minimal basal agar medium). Furthermore, on the second day the pH of bauxite residue treated samples under aerobic conditions was 6.4 and 6.1 for the anaerobic cultures with little changes thereafter (Figure 1). Results for the effects of the same nutrients on sample Nos. 5 and 6 (fresh bauxite

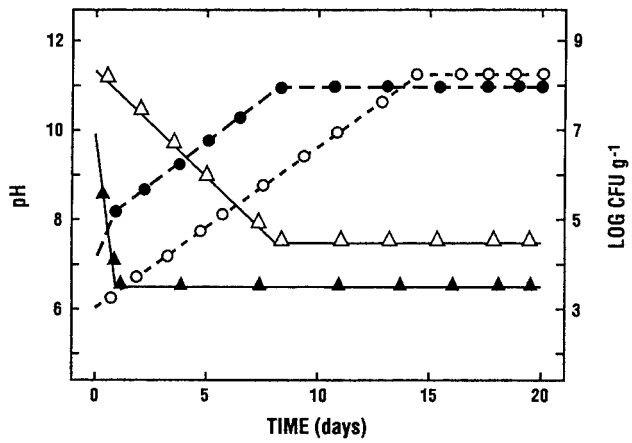

Figure 2 Effect of added nutrients on bacterial growth and pH of fresh bauxite residue slurry (sample No. 5) and lake water (sample No. 6) using the ratio of samples to medium as 2:1 (w/v). Sample No. 5 was obtained directly from the processing plant and No. 6 was secured from lake water of the impounded deposit site, where ○ represents growth (CFU) and △ pH of sample No. 5 and ● and ▲, respectively, for No. 6.

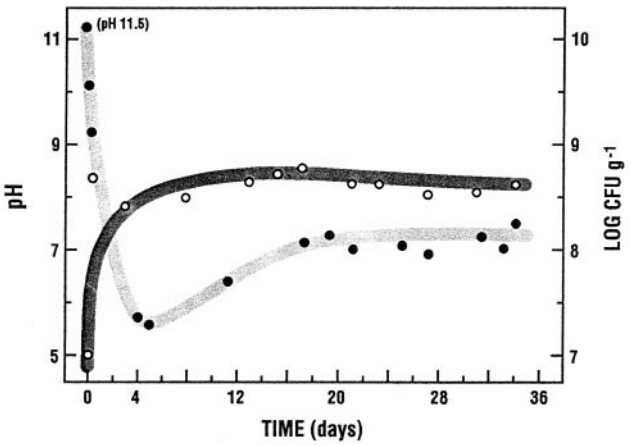

Figure 3 Effect of nonsterile Bermuda grass hay and other nutrients on bioremediation of bauxite residue using mixed sample Nos. 1–4 and the ratio of bauxite residue to medium was 3:1 (w/v). The nutrients used were (g l^{-1}): hay 40, peptone 5, yeast extract 0.5, $CaCO_3$ 0.1, glucose 2.5, and KH_2PO_4 2, where ○ denotes growth (CFU) and ● pH.

slurry and lake water, respectively) showed rapid aerobic growth during 15 days (Figure 2) and reached 10^8 and 2×10^8 CFU ml^{-1} for these samples, respectively, after 20 days incubation, and the pH was 7.5 and 6.3, respectively. Decreasing the peptone level in the medium from 5 to 2.5 g l^{-1} did not affect growth of either aerobic or anaerobic bacteria in the bauxite residue mixed sample Nos. 1–4 and the counts for both 10^8 CFU g^{-1} after 20 days and pH 7.0. Increasing the concentration of glucose to 50 g l^{-1} and changing the ratios of bauxite residue and medium to 1:1 (w/v) resulted in growth (10^9 CFU g^{-1}) and a stable pH of 7.0 during the entire 33-day incubation. When potassium phosphate (2 g l^{-1} water) was the only additive to the mixture of bauxite residue sample Nos. 1–4, there was no increase in microbial growth during the 3-week incubation and the pH was about 12. Fresh paper pulp (2 g) and phosphate (2 g l^{-1} water) were added to bauxite residue sample No. 3 (1:1, w/v) and incubated at 24°C without aeration. The promoters were added at intervals and the results showed that these additives had little influence on the microbial activities.

The level of the pulp paper was increased 10-fold to 20 g with sample No. 3 and the nutrients used were (g l^{-1} water): glucose 1, paper pulp 20, peptone 2.5, yeast extract 1, $CaCO_3$ 0.1, and KH_2PO_4 2, and promoters were added (3.0 ml with no paper pulp). The aerobic counts on the first day increased from 8×10^4 and 2×10^6 CFU g^{-1} after 10 days followed by a decline until the promoter was added that slightly enhanced the cell count and the pH was 9.1 with no further changes by the 50th day. This indicated that the medium used, containing higher level of fresh paper pulp, was of no value for inducing the desired microbial activities in the bauxite residue. The control experiment, which had bauxite residue sample No. 3 incubated with 20 g l^{-1} water and fresh paper pulp, showed no changes in number of CFU and pH. The experiment was repeated using 5-year-old paper pulp, but omitting both glucose and $CaCO_3$ and keeping the same ratio of bauxite residue (sample No. 3) to sterile media at 1:1 (w/v). The medium had (g l^{-1}): old paper pulp 20, peptone 2.5, yeast extract 1, KH_2PO_4 2. Promoter medium, without the paper pulp, was added to the mixture at intervals. Aerobic microbial growth increased and reached a maximum count of 10^7 CFU g^{-1} after 8 days followed by a gradual decline until the promoter was added, which elicited an increase in bacterial cell counts but only for a short duration. The pH fluctuated between 8.6 and 9.2 during the 20-day incubation at 24°C.

Hay analysis and bioremediation

Analysis of Bermuda grass showed (%): moisture 7.9, proteins 5, total sugar 2.6 and 5×10^6 CFU g^{-1}. Alfalfa hay had (%): moisture 10.8, proteins 12.6, total sugar 8.7 and counts of 8×10^5 CFU g^{-1} hay for both aerobic and anaerobic bacteria. Nonsterile ground Bermuda grass hay (40 g) with other nutrients (g l^{-1} water): peptone 5, yeast extract 0.5, $CaCO_3$ 0.3, glucose 2.5, KH_2PO_4 4, was used for bioremediation of bauxite residue (mixed sample Nos. 1–4), with the ratio 1:1 (w/v) for bauxite residue to medium. There was a rapid increase in the aerobic bacterial counts (Figure 3) reaching 6×10^8 CFU g^{-1} on the fourth day with a slight increase on the 16th day and no changes thereafter up to 34 days. The pH was 7.8 on the second day and 7.2 on the 20th day, with very little change thereafter. The control, which had water and an equal mixture of bauxite residue sample Nos. 1–4 showed no changes during 34 days of incubation. Ethanol and organic acids were present in both the filtrate and the solid particles of alfalfa hay-treated bauxite residue samples after 34 days incubation (Table 3).

Bioremediation of bauxite residue in column

The bauxite residue used in both columns was an equal weight mixture of sample Nos. 1–4. The first column had medium added on top of the column, the second had the medium mixed with bauxite residue and the medium had (g l^{-1}): glucose 30, peptone 2.5, yeast extract 1.5, $CaCO_3$ 0.1 and KH_2PO_4 2. The aerobic counts of samples from port No. 1 from the first column were 8×10^7 CFU g^{-1} on the second day and was almost the same after 36 days, whereas the bauxite residue from port No. 1 of the second column had 9×10^8 CFU g^{-1} on the second day followed by an increase of 10×10^{10} CFU g^{-1} on the 36th day after adding 275 ml promoter. The pH of samples from port 1 (first column) was 8.5 after the 10th day and 7.6 after 128 days compared to pH 7.3 on the 10th day (second column) with no changes thereafter to 128 days. Comparisons between port No. 1 of both columns indicated that the pH of 7.3 was due to the increased counts for both aerobic and anaerobic bacteria. Viable cell counts from port No. 4 (first column) after 30 days was 7×10^6 and 7×10^7 CFU g^{-1} for the

Table 3 Organic compounds (grams per liter) present in alfalfa hay-treated bauxite residue (filtrate and solid particles) for mixed sample Nos. 1–4. Results were reported as averages of three analyses (±standard deviation) of samples obtained on the 34th day of incubation at 24°C

Compound	Hay-treated bauxite residue (g l^{-1})	
	Filtrate	Solid
Ethanol	6.8±0.2	2.8±0.1
Lactic acid	12.2±0.5	5.2±0.2
Acetic acid	13.8+0.4	10.2±0.5
Propanoic acid	4.4±0.3	3.0±0.3
Butyric acid	1.0±0.2	0.6±0.1
2-Methyl propanoic acid	+[a]	+
Unidentified	+	+

[a]<0.2 g l^{-1}.

second column, and the pH was 6.6 for samples of both columns. Similar results were observed for port No. 5 and the viable aerobic counts from port No. 6 (first column) was 8×10^{7} and 9×10^{10} CFU g^{-1} (second column) on the 10th day, and the pH was 6.6 for samples from both columns on that day. The count of sample from port No. 7 (first column) was 6×10^{8} CFU g^{-1} and a pH of 7.6 and the from the second column was 9×10^{10} CFU g^{-1} and a pH of 7.0, with no changes after 170 days for both pH and bacterial cell counts. The bauxite residue samples from port No. 8 of the second column, but not of the first column, continued to maintain its high bacterial population (aerobic and anaerobic) and low pH during 15 days and thereafter to 140 days incubation. Conditions present at port Nos. 9 to 13 (both columns) indicated inhibition of aerobes, but the anaerobes yielded a count of 6×10^{10} CFU g^{-1} after 220 days. These results indicated that mixing the nutrients with bauxite residue prior to adding it to the second column allowed aerobic growth and movements of cells from the top to two thirds of column, and anaerobic bacterial cells to migrate to the bottom of column resulting in a stable neutral pH in treated bauxite residue. However, it took a longer time for bauxite residue for the first column to reach the activities of the second column indicating that the medium and bacteria were moving through the column but at a slow rate.

Identification of bacteria and scanning electron microscopy of bauxite residue

More than 150 bacterial cultures were isolated from the treated bauxite residue samples and most were identified to the genera: *Bacillus*, *Lactobacillus*, *Leuconostoc*, *Micrococcus*, *Staphylococcus*, *Pseudomonas*, *Flavobacterium*, *Enterobacter* and *Proteus*. However, many other isolates could not be identified. *Lactobacillus*, *Leuconostoc* and *Bacillus* were predominant, but all played a role in the physical properties and acid production leading to the bioremediation of the bauxite residue treated samples.

Scanning electron microscopy of bauxite residue before and after bioremediation (34 days) using an equal mixture of sample Nos. 1–4, and alfalfa hay treatment (Figure 4) showed that the particles in (A) for the untreated bauxite residue (control) were clumped together, and those of the hay-treated sample (B) were dispersed, indicating the desired physicochemical and other characteristics in the bauxite residue treated samples. Oval, spherical, and rod-shaped size particles (0.25 μm wide by 1 μm long) were observed in (B) as organized bacterial cell structures and microcolonies. Changes in both biological activities and the physicochemical properties of bauxite residue treated samples were due to the presence of large numbers of viable bacterial cells and the hay fragments were visible in/on the surface of bauxite residue treated samples.

Survival of plants and animals in bauxite residue

Data for survival of other biological systems in hay-treated bauxite residue (34 days with pH 7.6) showed that the red wigglers and night crawlers survived for more than 300 days, whereas in the control (untreated) they survived for only 6 to 24 h. Furthermore, the worms were able to "multiply" in hay-treated samples indicating a favorable environment. Various plants (poplar saplings), monkey grass (*Liriope muscari*), pampas grass (*Cortaderia seloana*), and two small cactus plants (*Mammillaria* sp.) grew in alfalfa hay-treated bauxite residue for 6–12 months. Pampas grass in the hay-treated samples was dormant during the winter season but initiated budding and growth during spring of the same year. The holly and loblolly pines (*Dracina marginata*) survived for 2–3 months in hay-treated bauxite residue, but died after exposure to an unexpected ice storm.

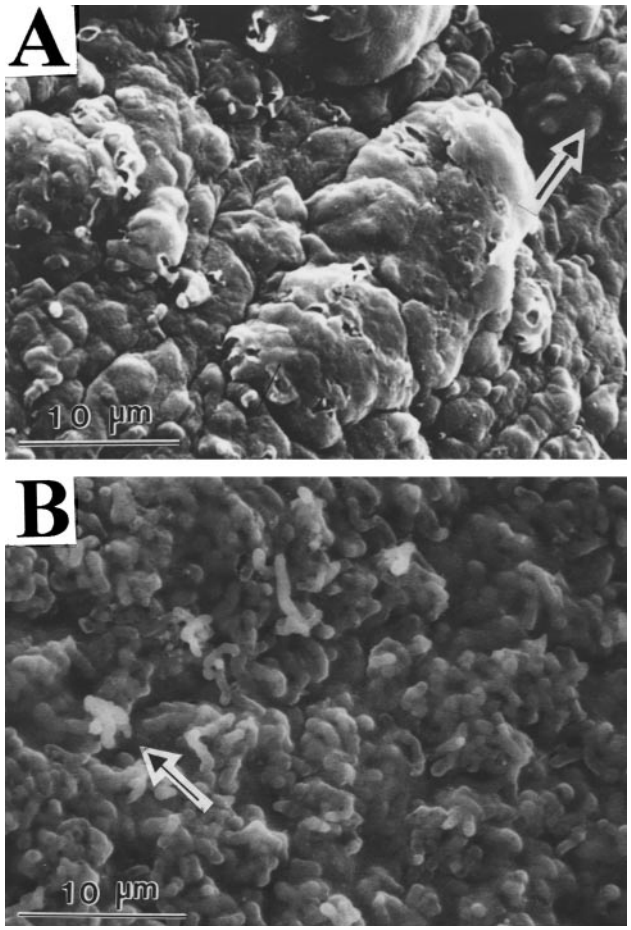

Figure 4 Scanning electron micrographs of (A) untreated (control) and (B) hay-treated bauxite residue. The treated sample was obtained after 34 days bioremediation using alfalfa hay on mixed bauxite residue sample Nos. 1–4. The control had large aggregated particles of bauxite residue clumped together as shown by the arrow whereas the hay-treated sample exhibited dispersed particles. Visible oval, sphere, rod-shaped structures and various microcolonies of different bacteria can be observed (bar = 10 μm).

Hossner (Alcoa residue dike vegetation demonstration study Alcoa Point Conmfort Operations, unpublished report, 1999) demonstrated the results of using Bermuda grass for bioremediation of bauxite residue on an experimental plot for deposit area at Point Comfort, TX, and reported that hay stabilized the surface of the bauxite residue deposit, minimized erosion and encouraged water filtration to remove the alkaline salt from the treated bauxite residue. Moreover, the hay-mulch treatment of the well-drained bauxite residue, allowed the establishment and growth of salt-tolerant vegetative plants.

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