Title: Non-structural Adhesives Requiring No VOCs

Project number PP1139

Final Report Prepared by

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I PROJECT TITLE: Non-structural adhesives requiring no VOCs

II PERFORMING ORGANIZATION: Montana Biotech Corporation, 1910-107 Lavington Court, Rock Hill, SC 29732

III PROJECT BACKGROUND

Polymeric adhesives are used in joining applications. Of the 5.6 billion pounds of adhesives used annually in the U.S., only 4% are based on natural materials. The feedstock for most adhesives comes from the petrochemical industry. Furthermore, 16% of adhesives include volatile organic compounds (VOCs), at rates as high as 80% (Nick, D.P. The North American Adhesive and Sealant Industry Convencion Annual Secciones, 2002. Posted at the following web site: http://www.ascouncil.org/news/ppt/dpna_asc_may2002.ppt). In sunlight, VOCs and nitrogen oxides produce ozone. Other VOC interactions contribute to the formation of photochemical smog.

Commonly used VOCs include toluene, methylethylketone and xylene. The 1996 Toxic Release Inventory indicates that 1.6 million pounds of VOCs were released through military use of solvent based adhesives. Civilian sector activities such as fiberboard manufacture and carpet installation release even more VOCs into the atmosphere.

Non-VOC adhesives would substantially reduce multiple air pollution sources.

In the study of natural adhesives, much research has focused on adhesion of mussels and barnacles. Although the compounds produced have demonstrated exceptional properties, their multi-component, complex nature makes them difficult to produce and prohibitively expensive.

A relatively unexplored source of natural adhesives was microorganisms. Montana Biotech Corp. has an in-house collection of microorganisms from a variety of habitats. This collection served as the source of microorganisms for this project.

IV OBJECTIVE

The overall objective was to identify microorganisms producing adhesives not requiring VOCs. Adhesive compounds were to be produced, partially purified, analyzed and tested on materials of interest to DoD, DOE and EPA.

Initially, the objective was to screen 500 organisms and then down select to 5 different adhesives. Purification, strength testing and chemical analyses were planned. The project identified more adhesive producing microorganisms than anticipated. It was determined that focusing on just one adhesive would permit earlier field demonstrations of success. The partial purification method turned

out to be adequate for most applications, complete purification being necessary only for medical applications. Therefore, attention was re-directed toward development of properties of interest to DoD.

V TECHNICAL APPROACH

The goal of this project was to screen 500 microorganisms from the Montana Biotech Corp. in-house collection for organisms producing adhesive materials. Organisms were to be grown in mini-fermenters. Polymeric materials were to be separated from spent media by an isopropyl alcohol precipitation. The adhesive strength of each preparation was to be determined by a flatwise tension test on aluminum adherends. Tensile strength of the biological material was to be compared with adhesives used by DoD for non-structural applications.

As compounds with significant adhesive strength were found, Tasks II and IV (preliminary and extensive purification) and Task V (chemical analyses) were to be initiated. The final task was to bring all the findings together and focus on small scale production of five adhesives.

Since adhesive materials were identified more quickly than originally anticipated and only partial purification was necessary, later tasks were adjusted to concentrate efforts on development of properties important to DoD applications.

In the following task by task discussions, the original task definitions (in italics) were modified to reflect the accelerated work plan.

Task I: Culture 500 microorganisms.

500 microorganisms were to be grown in YEP medium (yeast extract, peptone and dextrose) and in a defined salts medium in 250 or 500 ml mini-fermenters. The media were to be adjusted to within 0.2 pH units of the original habitat of the microorganism. Culture times generally were to be 2 to 4 days, with the shorter time required for organisms growing at 50 °C or above. Incubation temperatures were to be within 5-10 °C of the original habitat of each organism.

Previous studies indicated that not only medium composition, but also provision of a growth surface might have a significant impact on polymer production. Some microorganisms produce exopolymers constitutively, while others produce them only upon induction. Therefore, a mesh material was added to provide greater surface area for attached growth. Studies on alternative media formulations were added to this task.

Task II: Partially purify adhesive polymers.

A method was to be developed to isolate polymers from microbial cultures. Although structural components of these polymers were unknown and likely to

vary significantly from organism to organism, it was assumed that many of these microbial polymers would be polysaccharides and/or polypeptides. Cell-free spent media were to be treated with isopropyl alcohol. For protein polymers, ammonium sulfate fractionation was to be followed by HPLC. For polysaccharide polymers, proteins were to be removed. Again, HPLC was to be used for further purification.

Since the selected adhesive was determined to be a polysaccharide, it was not necessary to perform ammonium sulfate fractionation for protein polymers. However, extensive testing of precipitating solvents was added to this task.

Task III: Test adhesive strength.

Appropriate surface materials were to be determined and metrics obtained. An adhesion testing procedure was to be developed which would accommodate the strength range of the microbial preparations. Crude preparations from Task II were to be tested by the screening procedure. Those showing significant adhesive properties were to be subjected to Instron analysis. Each round of testing was to be used to down select first to 18 candidates and finally to the 5 best candidates covering a range of adhesive properties.

Initially, simple tension tests were to be used. More elaborate tests including shear and peel tests of various materials and under a range of environmental conditions were to be performed on samples selected by the initial screening.

Adjustments were made in coupon coatings (substitution of chemical agent resistant coating, CARC, for previously planned powder coating), but otherwise, adhesive strength tests remained essentially the same as those originally envisioned. Rather than attempt development of 5 different products simultaneously, one adhesive was selected as the focus of the later testing.

Although not a problem during screening, variability of test results became more apparent as smaller changes demanded more precision. A significant amount of time was spent on decreasing the variability by such techniques as surface preparation and regulating both the bond line thickness and the curing process. As the project moved closer to real-world applications, several sets of tests were done to compare the microbial adhesive with commercial products and with other natural materials.

To provide data obtained from an outside source, a CRDA was entered into with Redstone Arsenal. Alexander Steel and Robyn Sweitzer at Redstone Arsenal determined tensile strength and shear strength for both the parent and derivative adhesives in comparison with commercially available metrics. Single lap shear testing on plastics was also added to this task.

Task IV. Complete purification.

As the list of adhesive candidates was narrowed, the purification initiated in Task II was to be continued. Samples had to be purified to homogeneity for some of the chemical analysis work. It might not have been necessary to completely purify the final, marketable product but it was essential that all components were identified and tested singly. HPLC and GC-Mass Spectrometry, were to be used to purify each adhesive to homogeneity and to identify components.

The adhesive turned out to be largely a single polysaccharide and the extraction procedure proved to be quite selective, with 95-98% purity obtained on the first pass. Minor components did not seriously impact assays. Complete purification would only be necessary for medical applications. Rather than spending resources on purification not needed immediately, it was determined that a product less susceptible to the adverse effects of water was of more importance to DoD applications. Therefore, this task was re-written. First the structure of the adhesive was to be determined. Then appropriate derivatizations were to be made to alter polarity.

Task V. Perform chemical analysis.

Advanced analytical studies were to be conducted to further the understanding of these novel adhesives. Since these methods were time consuming and expensive, only samples displaying the best adhesive properties were to be subjected to this more advanced testing.

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) were to be used to give some insight into the molecular character and the adhesive performance of selected materials. Initially the ATR-FTIR work was to be used to quickly identify polysaccharide versus protein polymers. The more detailed work with this instrumentation would permit following absorption reactions and thus provide information on secondary structure. The ToF-SIMS would give a chemical fingerprint of each adhesive.

Extensive ATR-FTIR work was performed but ToF-SIMS work was largely replaced with tests more informative about polysaccharides. These included: molecular weight analysis, scanning calorimetry, nuclear magnetic resonance (NMR), colorimetric analysis and gas chromatography/mass spectrometry (GC/MS).

Task VI. Produce adhesive.

Selected organisms were to be grown in larger volume reactors to facilitate preparation of sufficient adhesive materials for outside testing.

This task was expanded. Originally, several 5-14 liter runs were planned. This provided a few hundred grams of adhesive, sufficient for laboratory scale adhesion tests. A larger scale production task was added as Task IX.

Task VII. Increase resistance to deleterious effects of water.

This task along with Tasks VIII and IX were added to the original work plan. Additional funding was requested from SERDP and this was used for these added tasks.

Although microbial adhesives were successfully isolated and adhesive strength was superior to the initial expectations, this group of adhesives had one significant problem in that they were water miscible to water soluble. In general, water solubility was proportional to the polarity of the molecule. To make that molecule more water resistant, polar moieties had to be derivatized with less polar groups.

Various derivatized materials were produced, converting hydroxyl groups to ethers or esters and incorporating cross linkable units such as aldehydes. Each product had different properties. Varying degrees of substitution of the hydroxyl groups were produced to afford the optimum balance between adhesive strength and water resistance. A kilogram of the acetyl derivative was prepared.

The CRDA worked out with Redstone Arsenal also covered environmental testing. The parent adhesive, the acetyl derivative and a control were subjected to a temperature cycling regimen at 85% humidity.

Task VIII. Material property characterization.

This task was added to the original work plan. Testing included thermal properties such as glass transition temperature and crystalline melting temperature as well as toxicolgy tests needed by potential users to determine conditions of use. In vitro toxicolgy tests provided an indication of mutagenicity and some idea of carcinogenicity. It also assured us there was no extreme toxicity. Dermal toxicity and sensitization which are of concern for a product such as this were not tested but as long as the adhesive was distributed strictly as a research material, the more expensive in vivo work was not needed at this time. Toxicon Laboratories was responsible for the testing.

Task IX. Scaled up production.

This task was added to the original work plan. This task involved scale up to 5000 liter fermentations, permitting production of over 50 kg of adhesive. The task was performed at Cathay Biotechnology using their pilot scale facilities. This company also has full scale production facilities which will permit a smooth transition from pilot scale to commercial production. The availability of the 50 kg

of polymer facilitated distribution to more potential users and testing on a larger scale.

It was also desired that sufficient derivative be prepared to permit distribution of samples. Polysciences, Inc. failed in its attempt to produce the derivative, so this work was also done at Cathay Biotechnology.

VI SUMMARY

The product developed under SERDP grant #DACA72-99-C-0013 is a natural polymer with adhesive activity. This polymer is made by a cost effective, microbial process. It is a pollution prevention material in that it is produced from a renewable resource, it is biodegradable and the final product does not release VOCs. This natural adhesive has a tensile strength of 500-1500 psi and shear strength of 250-800 psi on aluminum. It is very resistant to solvents such as jet fuel and d-limonene. As a wood adhesive, this polysaccharide has a shear strength of 1700 psi on hardwood. On softwood and manufactured woods such as particle board and medium density fiberboard, the adhesive bond is stronger than the wood adherends. Derivatized forms are resistant to the adverse effects of water. Both the parent and the acetyl derivative are able to withstand 85% humidity in a temperature cycling, environmental chamber.

VII PROJECT ACCOMPLISHMENTS

A provisional patent titled "Adhesives from Microorganisms" was filed in 2002. A full patent application has been filed. A paper accepted for publication covers much of the research data and is attached as part of Appendix A.

<u>Accomplishments by task:</u>

Task I: Culture 500 microorganisms.

Screening and selection: Microorganisms from the in-house culture collection were screened for the production of adhesive materials. More adhesive producing candidates were identified than originally anticipated. After screening 350 microorganisms, it was determined that a representative diversity of the Montana Biotech collection had been tested and plenty of adhesive samples were available. The 31 organisms identified as sources of adhesive materials are listed in Task III. It was decided to end the screening at this point and focus on organisms already selected. This more efficient approach freed up both technical time and funding that could be used for organism identification, characterization of the adhesive material and analytical tasks.

For those having studied adhesives from biological sources, the high strength adhesives obtained in this project were surprising. Three organisms commonly studied by others as a source of polymers (Pseudomonas fluorescens, P. fragi

and P. putida) were purchased and tested. No adhesive material was recovered. This indicates that the screening of a diversity of organism picked up species not dominating exopolymer production in biofilms, but with the ability to produce very strong adhesives.

After several rounds of testing involving both organism identification and tensile strength determinations, a single microorganism, 1222, was selected as the focus of the remaining work on this project.

Medium optimization: Initially, all organisms were grown in a defined salts medium and a yeast extract, peptone, dextrose (YEP) medium. The defined salts medium gave poor results and its use was discontinued. The YEP medium interfered with some analytical tests. Then it was found that the medium itself had some adhesive properties. Furthermore, a less expensive medium was necessary for a commercially viable product. Numerous media formulations were tested. One objective was to develop a medium with no complex components such as yeast extract. Although small amounts of these complex nutrients were beneficial, they were a potential source of variability when production was scaled up and laboratory grade complexes were no longer available. They also contributed to the stickiness of the product. Approximately 70 formulations and formulation modifications were tested. The formulation eventually selected was a modified version of a medium developed by Montana Biotech for production of glucose oxidase, thus the name GO8MOD5 (glucose oxidase medium number 8, modification number 5).

The single most costly component of the fermentation medium was the carbon source. Numerous low cost carbon sources were tested to provide a basis for selection of the carbon source for scaled up production. Maltodextrins, designated Maltrin 40, 100 and 180, and corn steep liquor were obtained from Grain Processing Corporation. Molasses from two different stages in a sugar processing plant were obtained from Brix-Berg. Fermentations using corn steep liquor and molasses, produced no adhesive. Use of maltodextrins at higher levels (10 gm/l or more), did result in adhesive production but the tensile strengths were generally below 100 psi.

Sucrose had been most successful so the monosaccharides making up sucrose, glucose and fructose, were tested separately. Use of either glucose or fructose resulted in no adhesive production. Surprisingly when both monosaccharides were added together, there was still no adhesive production. Only when the disaccharide, sucrose, was a part of the medium, was adhesive produced. It appeared that energy from the glucose-fructose bond was necessary for polymer formation. As little as 7 gm/l sucrose did result in adhesive production, but there was a direct correlation between the increase in the amount of sucrose added (up to 21 gm/l) and the amount of adhesive produced. Further increasing sucrose levels above 21 gm/l did not result in a significantly greater amount of

adhesive production in the 48 hour fermentation. It is possible that a longer fermentation would more efficiently utilize higher levels of sucrose.

Although phosphate was essential, there was evidence reported in the literature that phosphate was involved in regulation of exopolymer production. As phosphate was decreased, until a critical point was reached, polymer production was increased as part of a defensive mechanism. It was determined that the critical point was considerably lower than the amount previously used. Monobasic potassium phosphate was decreased from 0.8 gm/l to an optimum at 0.04 gm/l.

There was some evidence in the literature that certain polymers would be produced at a higher rate when calcium levels were increased. This did not prove to be the case for the present polymer. Also, in large scale production, the use of antifoam and tap water could be advantageous. Testing indicated that softened tap water was detrimental to production levels. The use of antifoam had no significant impact on production levels.

Test results indicated magnesium, iron and ammonium sulfate should not be changed from levels found in the original GO8 medium. A 75% reduction in citric acid was achieved without adversely affecting the polymer production. The added advantage to this change was that a reduction in the amount of sodium hydroxide was required to adjust the pH.

A suitable defined medium was eventually developed. That formulation was designated GO8MOD5.

Table 1: Components of medium GO8MOD5.

Medium components	gm/l
$K_2HPO_4 \cdot 3H_2O$	0.04
$MgSO_4 \cdot 7H_2O$	0.1
$FeSO_4 \cdot 7H_2O$	0.001
$(NH_4)_2SO_4$	0.2
Citric acid monohydrate	0.5
Sucrose	21.0
NaOH to adjust pH to 6.4	

Surface area: For screening of the first 250 microorganisms, a mesh material was provided for greater surface area of attached growth. As anticipated, some organisms did indeed grow attached to the mesh and produced copious amounts of exopolymer. However, that exopolymer did not prove to be a useful adhesive, so the mesh was left out of the mini-fermentors when the last 100 organisms were grown and was not present for re-growing previously selected organisms. Many of these fermentations did result in production of soluble exopolymer regardless of the presence or absence of the mesh.

Task II: Partially purify adhesive polymers.

Cell removal: The first purification issue was whether to separate the cells from the adhesive. Although the initial plan called for cell removal prior to adhesive precipitation, the cell volume was so small it was decided not to remove the cells during screening of the first 250 organisms. For screening of the last 100 organisms, cells were removed by centrifugation. At first it appeared this did make a difference, but after further experimentation, it became apparent that the improvement in adhesive activity was the result of changes in the medium rather than cell removal. The final procedure did not include cell removal. However, it was noted cells were readily removed by centrifugation if the final application called for a highly purified material.

Precipitating solvent: Several experiments were run to determine the optimum method for separation of the polymer from the spent medium. Ethyl lactate, acetone, ethanol, methanol, isopropyl alcohol and n-propyl alcohol were all found to be satisfactory. Although some varied with respect to the amount of material recovered and viscosity, the choice of the precipitating agent affects so many factors that a final choice will depend on the production facilities. The solvent will be recycled and the equipment for that process will affect the choice of solvent. Depending on shipping costs, it is possible the final product will be dried to a powder and reconstituted just prior to use.

In general, ethyl lactate produced one of the strongest adhesives but odor, weight and higher freezing point made it more difficult to work with in the laboratory. Alcohols were the least expensive to purchase, but recycling costs must be considered for large scale production. Location of the production facility will also influence the final choice. Ethyl lactate is produced at several plants in the Midwest so it would be significantly less expensive there. Engineering considerations such as distillation column design will be a cost to be factored into the choice of the precipitating agent. Product yield must also be considered. It may appear from the following table that the solvent used resulted in recovery of adhesives with differing tensile strengths. It appears more likely these observed differences are the result of viscosity variations. For example, when the microbial adhesive was made up to 50% solids, the average tensile strength from 10 tests was 855 psi. When the same material was diluted with water to 25% solids, the tensile strength average for 10 tests increased to greater than 991 psi.

Table 2: Effect of precipitating agent on yield and viscosity

Solvent (1:2)	Adhesive recovered gm/l	Average psi
Acetone	2.71	>1795
Isopropyl alcohol denatured	3.99	1640
Ethyl lactate	4.41	1427
Methanol	3.91	1346
Ethanol denatured	3.66	1136
Isopropyl alcohol certified	4.65	1005
Ethanol not denatured	3.83	978

Note: Averages of 5 tests are reported.

Solvent to spent medium ratio: The other processing parameter studied at length was the ratio between the precipitating agent and the spent medium. This varied with the specific precipitating agent. Ratios at intervals from 1:8 to 8:1 were tested for isopropyl alcohol. The optimum ratio was 1 part of spent medium to 1.5 parts of isopropyl alcohol. For ethanol, the optimum ratio was 1:2.

Until molecular weight determinations were done, the impact of changing the ratio on the final product was not recognized. There were 2 distinct molecular weight fractions found in the final product. One was over one million molecular weight while the other was in the range of 20,000-40,000 molecular weight. Initially, it was thought the lower molecular weight fraction would be the better adhesive since the smaller molecule would tend to give better solubility and would be more amenable to chemical manipulations. However, later it appeared this fraction was more prone to crystallinity. The higher molecular weight fraction could be made into a more homogeneous solution.

The distribution could be made to favor the higher molecular weight fraction by using isopropyl alcohol to precipitate the polymer. Ethanol recovered significant fractions of the lower molecular weight material. Changes in the medium and shorter culture times decreased the relative proportion of the higher molecular weight fraction. The final medium formulation and production procedure, resulted in a material that was predominantly of the higher molecular weight fraction.

Time for polymer precipitation: In large scale production, less time required for any process will translate into cost savings. Tests were set up using spent media from two different batches and isopropyl alcohol at a 1:1.5 ratio. It appeared that the 48 hour holding period was not necessary. In small volumes, as little as 2 hours was just as effective. In larger volumes, it may be expected that a longer cooling time will be necessary, simply to bring the temperature down. But once at -20 °C, the holding time was not critical.

Table 3: Precipitation time versus recovery

Time (hr)	Adhesive recovered (gm/l)
2	4.48
6	4.35
12	4.24
24	4.38
48	4.77

Procedure: The following procedure was worked out for lab scale production.

Grow culture 1222 in GO 8M OD5, pH 6.4, 1 m l air/m l m edium /m in, 36-48 hrs.
Harvest by transferring 100-150 m l to 500 m l centrifuge bottles.
Add cold (-20 °C) precipitating solvent (generally 100-300 m l).
Mix by inversion.
Cool entire solution to -20 °C.
Centrifuge at 1500 g for 10 m in.
Discard supernatant.
Invert bottles to drain for 10-15 m in.
Transfer precipitated adhesive to capped storage tubes.

This process recovered an adhesive product at approximately 40% solids. The material could be dried to a white powder and then reconstituted with water or water plus precipitating solvent prior to application. Approximately 25% solids resulted in the highest adhesive strength when bonding aluminum. As noted earlier, the cells could be removed by centrifugation prior to addition of the precipitating solvent.

Task III: Test adhesive strength.

Procedure: All screening tests made use of 2024 aluminum adherends. These were machined so that the surface area of the ends was exactly 0.25 square inches. Approximately half of the adherends were bare aluminum, while the other half were anodized. The surface coating did make a difference in adhesive strength, so care was taken to record the surface coating and to make sure that all samples to be compared, used the same coating. For the final year of the project, the anodized coating was removed and all tensile strength testing was done on bare aluminum. Adhesive was spread on one face of a pair of adherends. The pair was immediately held together by insertion into the Constant Force Fixture (CFF) calibrated to 20 psi. Samples were removed from the CFF after 1 hr. The curing process is discussed below. 3M products 1300 and 4799 were used as metrics throughout the project.

Test variability: Tests were generally run in at least triplicates; during the final year of the project sets of 5 or 10 adherends were used for each data point.

There was some concern that there was too much variability among replicates. The variability was not a problem during screening because the differences were so distinct. However, as the production method was refined, the differences between good and slightly less good adhesive were less distinct. Therefore, a study of the causes and possible remedies for the variability was undertaken.

Test equipment: Most of the testing during screening and early development was done on a Mark 10 at Montana Biotech and an Instron at Montana State University. Fifty sets of adherends were glued together from the same batch of microbial adhesive. Half were tested on the Mark 10 and half on the Instron at Montana State University. Although absolute numbers differed between the two instruments, it was determined that testing on the two instruments produced results of similar variability.

Adhesive: Twenty adherends glued with the commercially available adhesive that had been used as a control in this project, 3M 4799, were tested on the Instron. The variability of the commercial product was similar to the variability of the microbial adhesive.

Procedures: Surfaces were washed with acetone immediately before adhesive application. Also, excessive adhesive squeezed out at the bond line was removed. No significant improvement in reducing the variability was made.

For different test equipment, commercial versus microbial adhesives and changes in test procedures, the standard deviations were from 21% to 42% of the mean. It should be noted here this is not a problem unique to the microbial adhesive. In fact, this is a common topic of discussion at conferences relating to adhesives.

Surface preparations: It wasn't until the visit to the Redstone Arsenal laboratories that additional causes of variability were found. Redstone Arsenal personnel suggested use of disposable coupons. Previously, both bare and anodized coupons were washed and re-used. Exposure of the anodized coupons to hot water changed surface characteristics. Furthermore, it appeared that the incorrect anodization procedure had been applied originally so even the initial round of testing had been done on poorly prepared surfaces. This would contribute to differences observed between results using bare and anodized coupons.

Review of tests performed several months earlier at Redstone Arsenal, revealed that standard deviations were all less than 15% and most were less than 10% of the mean. This suggests that in addition to the obvious problems with coupons, there were problems with the test equipment. The Mark 10 was loaded manually, so more variations would be anticipated. The absolute values from the Mark 10 agreed more closely with the Redstone Arsenal results than with the

Montana State University Instron results. The Instron required extensive repairs and was not used during the final year and one half of the project.

Selection of microorganism: As noted in Task I, based on a summary of adhesive strengths, 31 organisms were selected for identification by sequencing and comparison of the 16S rRNA fractions. Duplicated organisms were to be eliminated. The laboratory performing the identification tests, MycoLogics, Inc., had a great deal of difficulty with the procedure. Five organisms were selected for further work but repeated testing resulted in different identifications each time.

Table 4: Adhesive strength of selected microorganisms.

Culture identification	Tensile strength, psi
54F	320
71X	470,580
92(D)F	894,756
125(A)F	440
154F	710
160F	747
165(C)X	282
195F	540,730
198(A)F	424,794
243(B)DYM	552,446
264(B)S-DYM	550
268(A)S-DYM	562
316 DUTT	610
444	632,730
476(WH)P3	660
555 P3	548
1204C	918
1222B	514,384
1223	492
1252	280,314
1272B	482
1306	650
1307	534
1320A	670
1320C	526
1324A	622
1359	240,340
1360	466,554
1381	818,806
180W	320,416
434 RED	766

The table above shows adhesion strength results from all 31 organisms. These are results of flatwise tension testing on 2024 aluminum adherends on the Mark 10. Load was manually applied on the Mark 10, accounting for some of the variation observed between different runs.

Even more of a problem than having the identifications change was the fact that the five selected organisms were supposed to be different organisms. It was later found that all five produced the same polymer and were closely related or duplicate species.

One organism, 1222B, was selected based on ease of growth, polymer production and strength of the adhesive produced. (Elsewhere in this report, this organism is identified simply as 1222). This was sent to Microbial Insights, Inc. for identification. The organism was identified with a 99% match. The identification and 16S sequence provided data needed for patent application

Test matrix: Once a single organism had been identified, a matrix was setup to ensure testing under a range of conditions. This matrix was designed largely by the Technical Advisory Committee to ensure all the data of interest to DoD would be obtained. The matrix was as follows:

1. <u>Screening</u>: 350 organisms were screened using bare aluminum - aluminum adherends in flatwise tension tests. From 31 organisms with adhesive activity, 18 unique organisms were selected based on organism identification. As discussed above, these later turned out not to be all unique organisms.

2. <u>Mechanical properties</u>: Three different types of tests were used to determine mechanical properties. These were flatwise tension tests joining anodized aluminum to anodized aluminum, the T-peel test joining aluminum and neoprene or nitrile rubber and the single lap shear test which joined coated aluminum to coated aluminum coupons. At the end of this testing, 5 organisms were to be selected. Test variability made selection difficult so organism identification was used to make the selections. Since sequence data were not available, it was only after the polymers were identified that it became apparent all five organisms produced the same polymer. It was also found that none of the polymers studied had good peel strength. The reason for this became apparent after the glass transition temperature had been determined. The adhesive was very brittle, so performed poorly with flexible materials in a peel test.

3. <u>Materials (peel)</u>: The effect of substrate variation was to be determined using 180 degree peel testing with 3 stiff and 2 pliable materials. These materials were epoxy graphite composite, urethane coated aluminum and powder coated aluminum as the base and nitrile rubber and neoprene as the flexible portion. It was found that the selected polymer adhered very poorly to both the nitrile rubber and the neoprene and showed no adhesive strength in the

peel tests, even using bare aluminum as the base. The problem was that the polymer was not flexible. After several trials, further peel tests were abandoned.

4. Materials (shear): A second test of substrate variation utilized the single lap shear method. Originally, the materials to be studied were epoxy graphite composite, epoxy coated aluminum, urethane coated aluminum and powder coated aluminum. Rather than having two epoxy surfaces, an anodized aluminum surface was substituted. It was also suggested that powder coating was of less interest to DoD, so chemical agent resistant coating (CARC) was tested in its place.

5. Environmental effects: Using the same polymer and metrics as above, the effect of temperature on adhesive preparations was to be determined. As work proceeded on the derivatized adhesive which was more water resistant, and because the water resistance issue became more important, it was decided to modify the environmental tests to include both temperature and humidity effects. The final procedure involved cycling between 30 and 60 °C at 85% humidity.

Plastics: Some plastics are fairly difficult to glue together, so a screening test was done using half a dozen different plastics. It was found the microbial polymer was effective for joining some plastics. Then a more extensive test was set up to include polycarbonate, epoxy glass, Fortron, ABS, TPX, DAP and PEEK.

Coupons and coatings: 2024 aluminum coupons were prepared for both shear and peel tests. Barry Avenue Plating applied all coatings. Coupons were divided into 4 groups as follows:

1. Anodized
2. Anodized and epoxy coated
3. Anodized, epoxy undercoat and urethane topcoat
4. Anodized, epoxy undercoat and CARC topcoat

Specifications were as follows:

1. Anodized: AMS 2471 sulfuric acid.
2. Epoxy: MIL 23377
3. Urethane: MIL PRF 85285
4. CARC: MIL-C-53039 or MIL-C-46168

Surface preparation and application of adhesives: Although Montana Biotech had facilities for tensile strength testing and access to additional equipment for tensile and shear testing, it was decided to send some samples to Redstone Arsenal for testing. This not only allowed testing on different equipment but also

involved people with specific expertise in adhesive strength testing. This outside testing thus stood as an independent analysis of the strength of the adhesive.

Under conditions of a CRDA, a materials scientist, Alexander Steel of Redstone Arsenal, traveled to Montana Biotech for several days. He taught Montana Biotech personnel how to prepare coupon surfaces and how to apply adhesive in compliance with MilSpecs.

Particular attention was paid to cleaning coupon surfaces ensuring the surface was fully wetted by the adhesive and completely covered the test area. Excess adhesive was removed. Some surfaces were cleaned with ethanol while others were cleaned with a "green" solvent, either Asahikin AK-225 or EnSolve. Comparing these results with those submitted to Redstone Arsenal earlier, it was apparent that surface preparation and more uniform application had increased shear strength.

Bond line thickness: A potential source of test variability was the bond line thickness. To determine this value for the natural adhesive, the tensile strength of adhesive between .003 and .037 cm thickness was tested. It was found to not vary significantly over that range. Microspheres were obtained and added to the adhesive to assure that bond line thickness would be maintained under a range of circumstances. Regardless of the amount of pressure applied, the microbeads maintain a constant distance between the two coupon surfaces and thus, a constant bond line thickness. Both offer a crush strength of 60,000 psi.

Two types of microbeads were obtained from 3M:

1. W-210 Zeeospheres composed of alkali alumina.
2. W-410 Zeeospheres composed of silicate ceramic.

By adding microbeads to the adhesive while it was still in its soluble form, thorough miking was assured. Microbeads were added at a rate of 1% and 10%. The average tensile strength from 5 tests is seen in the following table.

Table 5: Addition of Microbeads

Microbeads	Tensile strength psi
1% W-210	1077
10% W-210	920
1% W-410	884
10% W-410	820

Cure rate: Early testing indicated that curing was reliably completed in 10 days at room temperature. Experiments were performed at 50 °C to determine if more consistent results could be obtained at this elevated temperature. It was

determined that curing for 17 hrs at 50 °C resulted in the maximum tensile strength. Further raising the temperature to 55 °C, provided good results after 13 hours of drying.

Table 6: Effect of Time and Temperature on Cure Rate

Temperature °C	Time hours	Tensile strength psi
55	13	688
55	26	698
55	48	497
55	72	458
55	96	603
35	12	322
35	24	583
35	72	1006
35	168	831
35	240	773

Note: Tensile strength was the average of 5 tests.

Even at 55 °C, a cure time of 13 hrs was unacceptably long for many applications. Since the adhesive was recovered from aqueous media, water was entrapped in the adhesive. It was the slow evaporation of water from between two non-porous (aluminum) surfaces, that caused the very long cure time. It has been determined that the adhesive can tolerate temperatures at least to 121 °C for 25 minutes. For applications where heat can be used, this appears to be the most environmentally friendly approach. A hygroscopic solvent could be added, but many of these materials are classified as VOCs. The adhesive can be completely dried and shipped as a powder. However, it must be re-hydrated before application. It was found that for porous materials such as wood, the drying time was consistent with those of commercially available adhesives. For joining non-porous surfaces, the final procedure called for curing bonded materials at 35 °C for 10 days.

Rehydration: Once adhesive polymer subunits have been joined, for most non-pressure sensitive, commercially available adhesives, the process is not reversible. However, for the cured microbial adhesive under study here, water could be added to the broken bonds and the bond could be re-cured to the original strength.

Resistance to solvents: As a survey of potential applications was initiated, it was noted that many currently available products were not resistant to solvents. Testing of the microbial adhesive showed it was resistant to the effects of a variety of solvents. Adherends were joined with the microbial adhesive.

Following a 10 day cure at ambient temperature, they were soaked in solvent for 48 hours, and then dried for 1 hour prior to tensile strength testing. There was no loss of adhesive strength after soaking in jet fuel, d-limonene, methylethylketone or toluene. In fact, the following table indicates adhesive strength may have increased slightly. It is possible that these results reflect further removal of water from the adhesive.

Table 7: Resistance to solvents

Solvent	Tensile strength psi
Jet A	932
d-limonene	922
Methylethylketone	805
Toluene	766
Control-air	728

Adhesive strength tests at Redstone Arsenal: Under provisions of the CRDA mentioned earlier, all confirmatory tests were done at Redstone Arsenal. Expertise of personnel at Redstone Arsenal was very important in obtaining reliable data that would form the basis of future work and later marketing. This section includes summary tables of data obtained at Redstone Arsenal. Aluminum adherends with various coatings were joined with the parent adhesive, a methylated derivative or a 3M product designated 4799 and used as a metric.

Table 8: Shear strength on coated aluminum coupons in psi

Surface coating	Parent	Methyl derivative	3M 4799
Anodized	819	930	171
Epoxy	576	541	146
Urethane	0	0	185
CARC	651	533	185

Note: Shear strength was average of 5 tests.

The microbial adhesive performed well on anodized, epoxy and CARC coatings but poorly on urethane. For the next round of testing, the urethane surface was roughed using three strokes of 150 grit sandpaper, followed by wiping with solvent as usual.

Selected plastic coupons were also joined with the parent adhesive, methyl derivative (see Task VII) or the 3M product 4799 as the metric. The derivative generally adhered better to the plastics than the parent compound or the metric. TPX was tested with the other plastics, but none of the three adhesives bonded to this material.

Table 9: Shear strength on plastic in psi

	Parent	Methyl derivative	3M 4799
Epoxy glass	366	664	78
Fortron	—	519	83
DAP	188	87	112
ABS	152	309	65
PEEK	—	146	56
Polycarbonate	40	54	43

Note: Shear strength was result of a single test.

When 4 selected plastics were tested in the environmental chamber (described in more detail in Task VII), it was found that Epoxy glass was the only plastic that could be reliably adhered with the microbial adhesive. In all cases, shear strength was greater than 200 psi. There was some adhesion for the derivative on ABS. The adhesive failed to bond PEEK and Fortron in the environmental chamber.

Comparison with commercial products: Several commercially available adhesives were compared with the microbial adhesive. It should be noted that Elmer's is readily water soluble as is the parent microbial adhesive. Titebond II and Liquid nails came off after soaking in water. Crazy glue and the two 3M products were unaffected by soaking in water overnight.

Table 10: Comparison with commercial products

Adhesive	Tensile strength psi
Krazy glue	>1200
Elmer's	914
Montana Biotech	698
Titebond II	619
3M 1300	517
Liquid nails	290
3M 4799	161

Comparison with other natural polymers: Comparisons were made between the microbial adhesive and other natural polymers. A high degree of variability was found among various trials. It was determined that the percent solids was more critical than had been previously believed. An attempt was made to use preparations that were all at 25% solids. However, for some materials traditionally used as thickeners, it was not possible to use the materials at such a high percent solids. After dilution to the desired level, preparations were allowed to stand for 24 hours before use to allow complete solubilization. All adhesive

materials were applied to bare aluminum adherends, cured for 10 days at 35 °C and then tested for tensile strength on the Mark 10.

Table 11: Comparison of Montana Biotech Adhesive with Commercially Available Natural Polymers

Polymer	% solids	Tensile strength psi
Montana Biotech adhesive	25	>991
Montana Biotech adhesive	50	855
Corn starch, cooked	25	691
Dextran	25	479
CMC, sodium salt	17	193
Guargum	8	63
Xanthan gum	17	33
Alginic acid, sodium salt	17	20

Note: Tensile strength was the average of 10 tests.

Task IV. Complete purification.

Since complete purification was necessary only for medical applications, efforts were re-directed toward developing properties of more interest to DoD. Additional chemical analyses are reported under Task V and derivatization to improve water resistance is reported under Task VII.

Task V. Perform chemical analysis.

Initially, interference by components of the complex medium created significant problems when attempting to do ATR-FTIR tests. Following extensive modifications and re-testing, the defined medium discussed earlier was developed. In addition to resulting in the production of a superior adhesive, this defined medium did not interfere with various tests. For all tests reported in this section, microorganisms were grown in the GO8MOD5 medium. Assays were conducted at Montana State University.

Carbohydrate identification: Hydrolysis GC/MS to determine sugar repeat unit structure was unsuccessful and only accounted for low amounts of sample as carbohydrate. Since poor results with this method are known to occur when uronic acid units are present, the hydrolysis was repeated and a second attempt was made to identify the sugar units. Results were different but still accounted for only a small fraction of the total material present.

A different analytical approach was then used. Classical colorimetric analysis indicated the adhesive was 95-97% carbohydrate, 1-3% protein and 1% uronic

acid. Spectral patterns in both proton and carbon NMR were characteristic of polysaccharide.

ATR-FTIR: Using ATR-FTIR, the structure of the polysaccharide was determined. The structure is considered proprietary information and a patent to cover this information has been filed.

Molecular weight: Molecular weight analysis was done by size exclusion chromatography. Generally, molecular weights were in excess of one million although some products obtained prior to optimization of the medium and the process, had considerably lower molecular weights. These low molecular weight samples were readily soluble in water but insoluble in organic solvents, probably due to high crystallinity. The samples including low molecular weight materials showed three sharp peaks and one broad peak by size exclusion chromatography. The broad peak and two sharp peaks had molecular weights all below 40,000 while the last sharp peak had a molecular weight greater than one million. Changes in the growth conditions and changes in downstream processing parameters shifted the balance so that most of the material was found to have a molecular weight over one million and the material was more soluble in organic solvents used during derivatization.

Task VI. Small scale production.

Parameters for scale-up: Several runs were made to determine parameters associated with production of high levels of adhesive. The adhesive strength of the product was determined on samples taken at 24, 36 and 48 hours. Adhesive strength did not vary significantly among the time points but significantly less product was recovered per unit volume at 24 hours as compared with either 36 or 48 hours.

Data was acquired that was critical to scaled-up production. The first step was to collect data in the 5 and 14 liter laboratory fermenters. Four batches, each composed of 3 fermenters, were setup and run for 48 hours. All used GO8MOD5 medium. In the following table, the average (average of 3 fermenters in each run) absorbance at 600 nm, pH and reducing sugar concentration for each time point was recorded. In all cases the inoculum was 24 hours old.

Table 12: Fermentation data

Time hr	Abs_{600}	pH	Reducing sugar gm/l
6-7	.085	5.85	0.3
	.171	5.48	0.2
	.099	5.84	0.0
	.118	5.64	0.0
11-12	.437	4.91	0.3
	.627	5.40	3.3
	.424	5.51	2.0
	.474	5.45	2.0
22-26	.581	4.68	1.3
	.806	5.33	7.0
	.733	5.46	8.0
	.683	5.74	5.7
35-36	.658	4.54	8.3
	.890	4.93	8.0
	.742	5.49	9.0
	.741	5.52	7.0
48	.665	4.41	5.0
	.858	4.78	13.3
	.730	4.97	9.3
	.713	5.48	8.0

One unanticipated finding was the difference amongst the fermenters in terms of strength of the adhesive produced. This was consistent over the course of the run. For example, a fermenter producing an adhesive of higher strength at 24 hours, continued to produce higher strength adhesive at 36 and 48 hours. A repeat of this experiment again showed differences amongst the fermenters but in a different pattern, indicating that this was not caused by the fermenters themselves.

It was determined that production on a scale larger than the 5 and 14 liter laboratory fermenters was needed to push closer to field trials. Additional funding for scale up, toxicology and characterization was requested from SERDP and these funds were used to add Tasks VII-IX.

Task VII. Increase resistance to deleterious effects of water

Hydroxyls caused desirable and undesirable properties: The adhesive bond was adversely affected by water. It was theorized that hydroxyls on the polysaccharide backbone were determining both desirable and undesirable properties. The water solubility was proportional to the polarity of the molecule. To make that molecule more water resistant, polar moieties had to be derivatized

with less polar groups. Dealing with an adhesive, the problem was that the same polar groups interacting with water were also responsible for adhesive bond strength to polar surfaces such as aluminum. It became a matter of selecting a derivatization procedure which would cover a certain portion of the hydroxyls to inhibit water solubility, but leave enough hydroxyls exposed to maintain adhesive properties.

Derivatization: This task proved to be more difficult than anticipated. The insolubility of the adhesive in glacial acetic acid, dimethylsulfoxide, dimethylformamide, dimethylacetamide and dimethylacetamide/lithium chloride, posed a problem. It was eventually determined that the high and low molecular weight fractions had a striking difference in their ability to be derivatized. This was primarily a result of solubility in organic solvents. Whereas the low molecular weight product was only water soluble and chemical derivatization was largely unsuccessful, the high molecular weight product was sufficiently soluble in organic solvents to allow the desired modifications.

A series of methyl ether and acetate ester derivatives of varying degrees of substitution were prepared. Methylation was performed using dimsylpotassium base in dimethylsulfoxide and methyl iodide. Acetylations were performed with acetic anhydride in pyridine. Products were isolated either by precipitation into water (higher degrees of substitution), or dilution with water and dialysis if water soluble (lower degrees of substitution). Incorporation of methyl ether and acetate ester groups were confirmed by infrared and nuclear magnetic resonance spectroscopy.

As expected, water solubility of the products ranged from completely soluble to completely insoluble, but organic soluble. Adhesive strength and water resistance generally varied inversely as the degree of substitution was changed. Each was screened for adhesive strength on aluminum and for those that had retained adhesive strength, the materials were then tested for water resistance. Water resistance was tested in two ways, looking at the effects of both soaking in water and of high humidity. After curing, samples were soaked in 20 °C water or stored in a 75% humidity chamber at ambient temperature.

Table 13: Moisture Effects on Tensile Strength of Parent Adhesive and Derivatives

	Dry	0.5 hr Soak	2 hr Soak	4 hr Soak	7 Days 75%
Methylated -A	1546	228	374	260	
Methylated -B	>793		30	0	138
Acetylated -C	874	575	324		674
Acetylated -D	630	247		18	289
Parent-E	809	114	22	40	
Parent-F	829				91

NOTE: Dry refers to coupons after 7 day curing at 35 °C. Soak refers to coupons being cured for 7 days, then soaked in ambient temperature water and 75% refers to coupons being cured for 7 days, then held in a 75% humidity chamber. A and B are 2 different methylated derivative samples, C and D are 2 different acetylated derivative samples and Parent E and F are 2 different samples of the parent adhesive. Tensile strength (psi) was the average of 5 replicates.

It should be noted that the water insoluble, fully acetylated product could be cast into a tough film.

Thirty grams of the acetylated derivative were prepared at Polysciences, Inc. The product had a significant decrease in molecular weight. Polysciences failed in its attempt to produce a methylated derivative. To provide needed samples for potential users, one kilogram of acetylated adhesive was prepared by Cathay Biotechnology.

Effect of moisture on derivatives: The water stability of the acetylated derivative was compared with Titebond II, a commercially available adhesive designed for outdoor use, under a range of moisture conditions. As may be seen in the following table, the microbial adhesive maintained tensile strength slightly better than Titebond II after soaking or at 75% humidity. Results were similar for the two adhesives at 98% humidity.

Table 14: Comparison between acetylated derivative & Titebond II

	Treatment	Tensile strength psi
Acetylated	Dry	630
Acetylated	0.5 hr soak	406
Acetylated	5 hr soak	222
Acetylated	1 wk 75%	803
Acetylated	1 wk 98%	68
Titebond II	Dry	>736
Titebond II	0.5 hr soak	476
Titebond II	5 hr soak	63
Titebond II	1 wk 75%	261
Titebond II	1 wk 98%	48

Samples were prepared for testing in an environmental chamber at Redstone Arsenal. The temperature was cycled between 30 and 60 °C while the humidity was held around 85% . The program called for bringing the temperature to 30 °C and 85% relative humidity prior to initiation of the temperature cycling. The cycling started with a ramping up of the temperature to 60 °C over 2 hours, holding at that temperature for 4 hours, then ramping down to 30 °C over the next 2 hours and finally holding at that lower temperature for 4 hours. The cycle was repeated at the rate of 2 cycles per day for the course of 7 days. The specifications are from the MIL-STD-810E aggravated humidity cycle.

The parent adhesive performed better than had been anticipated. In fact, in many cases, the samples in the chamber had a higher shear strength than the controls. It is possible that the heating in the chamber served to drive out additional water, despite the fact that the samples had all been fully cured before shipment to Redstone Arsenal. Another unexpected result was that the parent adhesive not only often had greater shear strength than the derivative, but also suffered fewer breaks in transit or handling and fewer failures in the environmental chamber. The derivatization does cover some of the free hydroxyls important to adhesive properties. However, this is necessary to decrease interaction with water. The derivatives did perform better in static humidity chambers and when soaked, so this finding in the environmental chamber was not anticipated. Interestingly, the metric, always performed worse in the environmental chamber than the controls.

Most often, the failure was largely cohesive. The urethane coating showed 50/50 cohesive/adhesive failures. The CARC coating resulted in some mixed results. A summary of tests in the environmental chamber is in the following table.

Table 15: Results of single lap shear tests following incubation of cured samples in the environmental chamber.

Adhesive	Treatment	Failure	Shear strength (psi)	Number of tests (t); Number broken in transit (b); Number failed in chamber (f)
ANODIZED				
Parent	control	cohesive	392	t=5
Parent	chamber	cohesive	659	t=7,f=3
Acetyl	control	cohesive	490	t=4,b=1
Acetyl	chamber	adhesive	599	t=4,b=1
3M 4799	control	cohesive	171	t=5
3M 4799	chamber	cohesive	80	t=4,f=1
CARC				
Parent	control	50/50	252	t=4,b=1
Parent	chamber	cohesive	275	t=7,b=2,f=1
Acetyl	control	ad,slco	138	t=4,b=1
Acetyl	chamber	cohesive	303	t=3,b=1,f=6
3M 4799	control	cohesive	185	t=5
3M 4799	chamber	cohesive	95	t=5
EPOXY				
Parent	control	cohesive	360	t=1,b=5
Parent	chamber	cohesive	451	t=2,b=2
Acetyl	control	cohesive	499	t=3,b=2
Acetyl	chamber	adhesive	425	t=6,b=1,f=3
3M 4799	control	cohesive	146	t=5
3M 4799	chamber	cohesive	104	t=5
URETHANE				
Parent	control	50/50	339	t=2,b=9
Parent	chamber	50/50	377	t=4
Acetyl	control	50/50	134	t=2,b=6
Acetyl	chamber	50/50	207	t=4,f=3
3M 4799	control	cohesive	185	t=5
3M 4799	chamber	cohesive	109	t=5

Crosslinking: Another approach to reducing the solubility of a polymer is by crosslinking. Insolubility is generally observed at relatively low levels of crosslinking. Thus, the majority of the polarity of the structure is retained for adhesive purposes while a minor amount is utilized for water resistance. The mechanical properties of the adhesive are also affected by crosslinking. In general, incorporation of crosslinking into a polymer increases its modulus/strength and decreases its toughness/flexibility. The selected technique involved an aldehyde intermediate generated by partial periodate oxidation of the

polysaccharide to form a water insoluble carbazone. Although crosslinking afforded a water insoluble product, it was not water resistant and formed a product with increased stiffness and brittleness. Ultimately, a cross linkable adhesive may offer the best approach to a low VOC adhesive as a suitably designed water soluble or even neat precursor could be cured to a water insoluble and water resistant product. However, at this point, the acetylated and methylated routes offer the best option.

Task VIII: Material property characterization

Description: The adhesive, in both parent and derivative forms, was white and sticky to touch. The viscosity was similar to putty although this varied with the ratio and specific solvent used in the precipitation. Before application, the viscosity was decreased. Usually mixing with equal parts of water or water plus solvent, provided a readily spreadable adhesive. For some applications, one part of adhesive was mixed with up to 3 parts of water.

The adhesive could be dried to a fluffy, white powder by freeze drying, drying under a vacuum at elevated temperature or simple evaporation. It was reconstituted with water or water plus solvent to the desired viscosity

Heat stability: Unlike most biological products, the adhesive had good heat stability. After samples had been glued and cured, they could be autoclaved (121 °C, 15 psi, 25 minutes). If they were allowed to cure again (moisture in the autoclave disrupted the bonding), they retained the original adhesive strength. The autoclaved samples read an average of 811 psi and the control that had not been autoclaved read an average of 730 in the tensile strength test on aluminum.

The melting point and the glass transition temperatures were determined by differential scanning calorimetry (DSC). The scan was started at 25-150 °C at 10 °C/min, cooled to room temp, and then rescanned from 25-250 °C at 10 °C/min. The first scan was performed to volatilize residual water. The T_g (glass transition temperature) was at 138 °C. A large endotherm occurred at about 225 °C, indicating the T_m, or melting transition temperature. This indicated this material was semicrystalline.

Toxicology: Two toxicology tests were performed on the parent compound. It was found to be non-cytotoxic, by the Agar Diffusion Test, ISO 10993. Endotoxin was found present in the preparation. Dilutions were done out to 1:256 and the corrected Endotoxin Units/ml was reported as greater than 615. By way of comparison, xanthan gum, a commercially available microbially produced polysaccharide used as a food additive, was also tested and found to have greater than 1280 Endotoxin Units/ml. Compared to the classic exotoxins of bacteria, endotoxins are less potent and less specific in their action, since they do not act enzymatically. Endotoxins remain associated with the cell wall until disintegration of the bacteria. For medical applications, removal of the cells prior

to adhesive precipitation would be expected to remove the endotoxins. They are not anticipated to present problems in other applications.

Task IX: Scaled up production

Task IX was added to produce the adhesive at pilot scale. These 5000 liter fermenters served to demonstrate the feasibility of full scale production, provide data on production costs and produce sufficient material to distribute to potential users.

Following confirmation of the procedures at the research laboratories of Cathay Biotechnology using locally obtained medium components, two 5000 liter fermentations were successfully completed at the Cathay Biotechnology pilot plant. About 50 kg of adhesive was produced and this was the material provided as samples for potential users.

The pilot plant material was tested for adhesive strength and molecular weight. A slight decrease in molecular weight was noted, but this was likely due to the use of ethanol as the precipitating agent rather than isopropyl alcohol which had more frequently been used at Montana Biotech. The adhesive strength was the same as obtained from materials produced in smaller volumes. The cost of production at the 5000 liter scale was $20/kg.

VIII CONCLUSIONS

Utility: A microbial polymer was found which could form the core of a useful adhesive. Unlike petrochemical based adhesives, this adhesive is made from a renewable resource, sugar. As a natural microbial product, it is biodegradable. It is also non-cytotoxic.

This "green" adhesive has good tensile strength of 500-1500 psi on aluminum with various surface finishes. In fact, the adhesive bond is stronger than medium density fiberboard, particleboard and a softwood, fir. The microbial adhesive is particularly attractive for assemblies where a temporary bond is needed; after use, the bonding material can be washed off. Another short term bonding situation is for certain pallets that will be subject to burning. Petrochemical adhesives release toxic materials upon ignition, but this natural adhesive can be safely incinerated. The resistance to solvents by this adhesive suggests use in repairs, where solvents are in use for cleaning but will damage petrochemical based adhesives. A major problem for recycling paper is clumping of adhesives; this microbial adhesive eliminates the problem making it of value for compostable paper products.

The microbial polymer is not restricted to use as an adhesive, but may also be used in medical, food and feed, and remediation applications. Polysaccharides have been studied for applications in many industries. The specific polymer

developed here already has demonstrated value as a processing aid, a hypocholesterolemic agent, antitumor drug, in wound repair, as a biodegradable film for food packaging and as a low calorie sugar substitute. Until now, those applications had been unrealized largely because the polymer was so expensive ($100/gm). The low cost production method developed here, will make these uses economically favorable.

Economic feasibility: Perhaps the single most important development to come out of the research reported on here was the ability to produce this microbial polymer in a 5000 liter fermenter. This polysaccharide is currently sold for $100 per gram, a price that prohibits its use for any application outside of limited laboratory studies. Media and processing procedures developed during this SERDP funded project facilitated production on a scale large enough to bring the price within range of other types of microbial polymers, $20 per kg. This 5000 fold reduction ($100 to $0.02/gm) in price opens up numerous possibilities, although the price remains marginal for application as a commodity adhesive. A further price reduction can be expected once production moves from the pilot scale to full scale.

General attractiveness: In the very competitive marketplace, a new adhesive must have properties dramatically superior to current products to result in a change to the new adhesive. One reason most companies offer a family of adhesives is that no single material can meet all the criteria for numerous adhesive applications. The microbial adhesive is marginally price competitive as a general commodity adhesive and it has properties that preclude its use in some situations. However, it does have strong points which make it an attractive product in specific markets. Its "green" attributes (renewable feedstock, biodegradable final product, no VOC release during cure) are its strongest points. Another particular advantage of this adhesive is that it may be stored for extended periods in the form of a powder. With the price of petroleum rising rapidly, the price of this sugar-based product may soon be very attractive, even before the economy of scale begins to have a significant impact. Multiple applications not only as an adhesive but also in the food and feed industries and in medicine, is another point that makes this material particularly attractive.

IX TRANSITION PLAN

The transition from basic research to the marketplace has been approached from several directions. Additional grants were designed to look at problem areas. A concentrated effort was directed toward getting the word out about this new product. An alliance was formed with another company already producing other types of microbial polymers, bringing the production facilities, sales and marketing skills needed to get this product to the marketplace.

Proposals: A SBIR proposal was submitted in January 2003 to DOE by Montana Biotech. The proposed work will test the hypothesis that concurrent production of the adhesive polymer and ethanol will assure the economic viability of the polymer as well as a non-petroleum energy source. The polymer production includes the use of ethanol and excess, fuel grade ethanol can be sold as a separate product.

Another proposal has been submitted for the purpose of further examining alternative uses of this polymer, of specific interest to DoD. Additional proposals are in the planning stages for submission to NSF and EPA, also to look at alternative applications for this adhesive.

Conferences: During the wrap up meeting for the SERDP funded project held at Wright Patterson Air Force Base in August, it was determined that one of the most important issues not yet sufficiently addressed, was to get the word out to potential end users and potential partners. This effort took several forms, focusing on trade shows and direct contacts.

A Montana Biotech representative attended the following conferences (presentations at the two final events have been accepted):

Adhesives from Natural Sources Workshop, Cleveland, OH, September 2002.

Adhesive and Sealant Council, Pittsburgh, PA, October, 2002, booth presentation.

Partners in Environmental Technolbgy, Washington, DC, December 2002, poster presentation.

Adhesion Society Annual Meeting, Myrtle Beach, SC, February 23, 2003, oral presentation.

European Congress for Raw Materials for Adhesive and Sealant Industry and European Coatings Show, Nurnberg, Germany, April 9-10, 2003, oral and booth presentation.

7th Annual Green Chemistry and Engineering Conference (ACS and NSF), Washington, DC, June 23-26, 2003, oral presentation.

Web site: A web site was setup and may be accessed at http://natural-polymer.com

Mailing list: A mailing list of about 300 companies was prepared. A one page handout was mailed to all companies on the mailing list. There have been a number of responses and samples have been submitted for testing at potential user's laboratories.

Presentations on site for potential users: Montana Biotech received invitations to make a presentation at two private companies. One of these companies was 3M; a secrecy agreement prevents release of the second name. There was significant interest in both cases and discussions are continuing.

DoD interests: Significant progress has been made for applications in the private sector. Although some may be of benefit for DoD, a search for specific applications by DoD is ongoing. The following ideas have been discussed with DoD personnel. Follow up on these ideas will be pursued in the coming months.

1. Repair: Two properties of the microbial adhesive make it of particular value for repairs. In situations where a temporary bond is needed, the microbial adhesive can be used and then readily removed with water. The resistance to solvents is also useful in places where solvents are being used for cleaning. Unlike petrochemical based adhesives, this adhesive is resistant to solvents.

2. Ammunition/missiles: Missiles have parts sealed under inert gas so that water solubility is not a problem, but emission of VOCs would be harmful. This non-VOC adhesive may find application in this setting. As a "green" adhesive, the microbial product has potential in programs where biodegradation is important.

3. Pallets/manufactured wood products: Certain pallets are burned after use. A polysaccharide based adhesive will not release the toxic fumes found in more commonly used adhesives.

Alliance for production, marketing and sales: Most importantly for a transition to the marketplace, production, sales and marketing are available for immediate implementation as a result of an alliance with Cathay Biotechnology. This company has successfully produced 50 kg of the microbial polymer in its pilot plant. They also have a large scale production facility where they produce other microbial polymers. The Montana Biotech polymer will fit into their product line which is distributed world-wide. The alliance between Cathay Biotechnology and Montana Biotech takes advantage of production and distribution facilities that are already in place and are directed toward many of the same customers who would be potential users of the Montana Biotech polymer. This alliance also makes provision for royalty-free use of the polymer by DoD.

X RECOMMENDATIONS

Polysaccharides represent a class of materials of growing interest to many industries. Montana Biotech will continue a very active search for specific uses by DoD and getting the word out to potential users.

Technically, there are two areas where resolution of a problem would be of significant benefit. (1) Derivatization by a chemical process is very expensive. Numerous microorganisms are known to perform both acetylations and methylations. It is recommended that a research project be set up to find an organism that is able to derivatize this polymer. (2) Water susceptibility may also be overcome by another approach. The current research used one screening method to look at a large number of organisms for an adhesive material. Now that it has been established there are many more microbial adhesives than originally anticipated, a second approach would be to use multiple screening methods to look at a small number of organisms for an adhesive material. This approach would ensure recovery of adhesives with different types of properties, including resistance to water.

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The Technical Advisory Committee provided guidance throughout the project and Montana Biotech wishes to acknowledge the value of the input of all members. Alan Fletcher and James Tira traveled to all meetings and gave frequent assistance during the entire project. Others who served as technical advisors at critical points were Joseph Brescia, William DePiero, Francis Sullivan, James Mazza, Georgette Gaskin, and Matthew Tillman. Their advice continues to be most helpful as specific DoD applications are pursued.

Numerous people participated in the project as subcontractors. Their roles are acknowledged in appropriate sections of this report. Two groups who made substantial contributions were personnel from Montana State University and Redstone Arsenal. Anthony Haag, Peter Suci and Gill Geesey from Montana State University were responsible for much of the analytical work, the derivatization and assistance with presentations. Redstone Arsenal personnel Alexander Steel and Robyn Sweitzer played a vital teaching role in surface preparation and adhesive application as well as performing all of the shear tests and confirmation of tensile strength tests. Mark Feathers and Steven Carr from Redstone Arsenal and Dan Swanson from the Montana State University TechLink Center cooperated in the preparation and realization of the CRDA which provided the framework for the interaction with Redstone Arsenal.

APPENDIX A

Peer-reviewed Papers.

Adhesive Produced by Microorganisms, J. Combie, A. Haag, P. Suci, G. Geesey, accepted for publication by ACS. This paper has been attached to the end of this report.

Technical Reports.

SERDP Project Taps Potential for Non-VOC Adhesives. SERDP/ESTCP Information Bulletin, No. 8, Spring 2001.

Adhesives from Nature: VOC-free, Non-structural Adhesives. (Robyn Sweitzer, Titan Systems Corp., EELO, AMSAM-EN), Hazardous Technical Information Services (Vol. 12, No. 4), a publication from the Defense Supply Center Richmond.

Conference/Symposium Proceedings Papers.

Adhesives from Bacteria, oral presentation. Paper published in Proceedings, 25th Annual Meeting of the Adhesion Society, Orlando, FL, February, 2002.

Microbial Adhesive - Properties and Production, oral presentation. Paper published in Proceedings, 26th Annual Meeting of the Adhesion Society, Myrtle Beach, SC, February, 2003.

Adhesive from Microbial Polymer, oral presentation. Paper published in Proceedings, 3rd European Congress on Adhesive and Sealant Raw Materials, Nurnberg, Germany, April, 2003.

Published Technical Abstracts.

Non-structural Adhesives from Extremophilic Microorganisms (oral presentation): Technical Advisory Committee Meeting, Center for Biofilm Engineering, Bozeman, MT February 3-4, 2000.

Non-structural Adhesives Requiring no VOCs (poster presentation): Opportunities 2000 Small Business Conference, Billings, MT, February 23, 2000.

Diversity of Materials from Extremophiles (poster presentation): IEEE, Big Sky, MT, March 2000.

Non-structural Adhesives from Extremophilic Microorganisms (oral presentation): Army Environment Quality (EQ) Information Exchange Meeting, Williamsburg, VA, July 18-20, 2000.

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Non-structural Adhesives Requiring no VOCs (oral and poster presentations): Partners in Environmental Technology, Nov 28-30, 2000.

Bacterial Adhesives with no VOCs (poster presentation): Partners in Environmental Technology, Washington, DC, November 27-29, 2001.

Microbial Adhesive (poster presentation): 7th Pacific Polymer Conference, Oaxaca, Mexico, December 3-7, 2001.

Novel VOC-free Adhesives from Microorganisms (oral presentation): 12th Annual International Workshop on Solvent Substitution and the Elimination of Toxic Substances and Emissions, Scottsdale, AZ, December 10-13, 2001.

Adhesives from Natural Products - Multifunctional Polymer (booth presentation): The Adhesive and Sealant Council 2002 Fall Convention and Exposition, Pittsburgh, PA, October 15, 2002.

Bacterial Adhesives with no VOCs (poster presentation) and abstract published by Partners in Environmental Technology, Washington, DC, December, 2002.

Multifunctional Polymer Produced by Bacterial Fermentation (oral presentation accepted): 7th Annual Green Chemistry & Engineering Conference, Washington, DC, June 23-26, 2003.

Adhesive Produced by Microorganisms

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A water-based adhesive from non-petrochemical feedstock was produced by an efficient microbial fermentation process. The adhesive was susceptible to water but very resistant to solvents such as jet fuel. After curing, the adhesive could be re-moistened and used again to bond surfaces together. Shear strength on anodized aluminum averaged 819 psi and tensile strength ranged from 500 to 1500 psi depending on the substrate and production method. The parent adhesive was modified to several water resistant forms which maintained good adhesive strength.

Adhesives often include toxic volatile organic compounds (VOCs) such as toluene, methylethylketone, and xylene. In sunlight, VOCs and nitrogen oxides produce ozone. Other VOC interactions contribute to the formation of photochemical smog. At elevated levels, VOCs present both a health and fire hazard and degrade the environment. The US used 5.6 billion pounds of adhesives in 2001. Sixteen percent of these were solvent based systems (1), releasing several hundred million pounds of VOCs into the atmosphere.

The search for environmentally compatible adhesives has turned to such examples of nature as the tenacious adherence of barnacles and mussels (2). Although the properties have indeed been spectacular, production of these adhesives on a commercial scale has been problematic.

It is well known that many microorganisms produce extracellular polymeric substances (EPS) with adhesive properties (3,4,5,6). The advantage of using microorganisms over mollusks is that the technology for commercial scale production of microorganisms is well established. The work reported here focuses on use of readily-grown microorganisms as a source of novel, environmentally friendly adhesives at a reasonable cost.

Experimental

Screening of Microorganisms

350 isolates from an in-house culture collection were raised in liquid culture and screened for those producing adhesive EPS. Adhesives were ranked by tensile strength on bare aluminum.

Optimized Production Process

Microorganisms were grown in the following liquid medium (gm/l): 0.08 $K_2HPO_4 \cdot 3H_2O$, 0.1 $MgSO_4 \cdot 7H_2O$, 0.001 $FeSO_4 \cdot 7H_2O$, 21 sucrose, 0.2 $(NH_4)_2SO_4$, 2.0 citric acid. The pH of the medium was adjusted to 6.4 with 10 N NaOH prior to sterilization. Following 2 days of growth at 35 °C, spent medium was mixed with -20 °C solvent such as isopropyl alcohol, causing the adhesive to precipitate out of solution. The mixture was centrifuged at 1500 g for 10 min, the supernatant was discarded and the putty-like adhesive collected from the bottom of the container.

Adhesion Testing Procedure

Tensile Strength

For determination of tensile strength, a pair of 2024 bare aluminum cylindrical adherends with one quarter square inch surface area and 1 inch long, were joined with the test adhesive. The two cylindrical adherends were held together in a constant force fixture for 1 hour, to allow the adhesive to set up. Curing was by drying so the cure time varied with the precipitating solvent and the adhesive concentration.

After curing, the adherends were pulled apart using a manually operated Mark 10, Model BGI, a force measuring instrument. The tensile strength was generally determined on 5 pairs of adherends and the average recorded.

For solvent resistance determination, after curing the adherends were submerged in the solvent to be tested for 48 hours. The adherends were then pulled apart as described above.

Shear Strength

2024 aluminum T3 coupons, 1 inch wide and 4 inches long, were coated at Barry Avenue Plating, Los Angeles, CA. Coupons were divided into 3 groups as follows: (1) Anodized, (2) Anodized and epoxy coated, and (3) Anodized, epoxy undercoat and CARC topcoat Specifications were as follows:

- Anodized: AMS 2471 sulfuric acid. This specification includes a hot water seal which seals the aluminum but makes a poor surface for adhesives. Through a misunderstanding, this hot water seal was included. Therefore, adhesion to these surfaces would be expected to be significantly less as compared with alternative anodization methods.
- Epoxy: MIL-P-23377
- CARC (Chemical Agent Resistant Coating): MIL-C-53039

Particular attention was paid to cleaning coupon surfaces ensuring the surface was fully wetted by the adhesive and completely covered the test area. Surfaces were cleaned with ethanol or EnSolv (n-propyl bromide, Enviro Tech, Melrose Park, IL). Adhesive was applied to a 1 inch by 0.5 inch area of one coupon and the second coupon overlapped that area. Coupons were clamped together for 1 hour to allow the adhesive to set up.

Shear strength testing was done on an Instron Universal Testing Instrument (Screw Drive), Model Number TTC with an Instron Tensile Load Cell, Model D, 1000 pound full scale range. ASTM Specification D1002-99 was followed. All shear strength testing was performed at Redstone Arsenal by Alexander Steel.

Water Resistant Derivatives

A series of methyl ether and acetate ester derivatives of varying degrees of substitution were prepared. Methylation was performed under anhydrous conditions using dimsylpotassium base in dimethylsulfoxide and methyl iodide. Acetylations were performed with acetic anhydride in pyridine which contained a small amount of water to aid solubilization of the starting material. Products were isolated either by precipitation into water (higher degrees of substitution), or dilution with water and dialysis if water soluble (lower degrees of substitution). Incorporation of methyl ether and acetate ester groups were confirmed by infrared and nuclear magnetic resonance spectroscopy.

The water solubility of the products ranged from completely soluble to a highly swollen gel to completely insoluble (but organic soluble). Each was screened for adhesive strength on aluminum and if favorable, the materials were then tested for water resistance after curing by soaking in water at 20 °C and by storage in a 75% humidity chamber at ambient temperature.

Results and Discussion

Screening of Microorganisms

A single organism was selected as the focus of the majority of the work reported on here, based on results of flatwise tension tests. The organism has not yet been identified. It should be noted that organisms used by others looking for microbial adhesives such as Pseudomonas fluorescens (4), P. fragi (7), and P. putida (8) produced no adhesive under our conditions.

Production Process

Medium

Numerous experiments were conducted to optimize the composition of the growth medium. Twelve different media with up to eight modifications of each medium were tested. The organism grew on most of the media and in only a few cases, was no adhesive produced. Adhesive production did require the presence of sucrose; three sources of molasses, glucose, fructose and maltose could not be substituted. All carbon sources tested permitted growth of the microorganism but use of any carbon source other than sucrose resulted in recovery of no adhesive. Only a few nitrogen sources were tested, but it was found that substitution of nitrate for ammonium ion resulted in absence of recoverable amounts of adhesive. Removal of iron decreased the yield of adhesive by about half, but amounts ranging from 0.001 to 0.020 gm/l 0.001 $FeSO_4 \cdot 7H_2O$ appeared to have little impact on the production. When magnesium was absent, the microorganism did not grow, but all levels tested between 0.1 and 0.8 gm/l $MgSO_4 \cdot 7H_2O$ had little impact on adhesive production.

It is known that stress can increase EPS production. Therefore several stress factors were introduced into the growth medium. Although phosphate was needed, the less phosphate available (down to 0.01 gm/l $K_2HPO_4 \cdot 3H_2O$), the greater the yield of adhesive. Since this was a poorly buffered medium, it was found that 0.04-0.08 gm/l $K_2HPO_4 \cdot 3H_2O$ resulted in more reliable production.

Another set of experiments was done to compare the effect of placing screens made out of autoclavable plastic, aluminum or copper in the fermenters. It was anticipated that the stress of high copper levels might increase the polymer production. It was also theorized that providing greater surface area might also increase the amount of polymer produced. No significant effects were seen and this line of the investigation was dropped.

Precipitating solvent

Since the adhesive is soluble in the aqueous medium, some method for recovering the adhesive had to be employed. Both solvents and ammonium sulfate were tested. All solvents tested that were miscible with water, were satisfactory. Although minor differences were seen in the yield, solvent cost and the ease of recycling would need to be considered before a choice could be made. Satisfactory precipitating solvents were methanol, ethanol, isopropyl alcohol, acetone and ethyl lactate (Table I).

Table I. Effect of Precipitating Solvent on Adhesive Yield and Tensile Strength

Solvent	Yield gm/l	Tensile Strength psi
Methanol	6.47	1346
Ethanol (denatured)	6.87	1136
Ethanol (not denatured)	7.47	978
Isopropyl alcohol (reagent)	7.54	1640
Isopropyl alcohol (certified)	7.60	1005
Acetone	7.34	>1795
Ethyl lactate	9.14	1427

NOTE: Tensile strength is average of 5 replicates. One part of spent medium was mixed with two parts of solvent. Yield is wet weight.

Use of precipitating solvents that were not miscible with water did not result in recovery of adhesive. Those tested in this category were ethyl acetate, hexane, n-butanol and sec-butanol. Precipitation with 10, 40 and 70% ammonium sulfate was also unsuccessful.

Several experiments were done to determine the optimum ratio of the precipitating solvent to the spent medium. Ratios tested ranged from 1 part spent medium to one-eighth part solvent through 1 part spent medium to eight parts solvent. Although the optimum varied slightly with the solvent, the optimum was generally in the range of 1:1 to 1:2 spent medium to solvent.

Appearance

The adhesive, in both parent and derivative forms, is white and sticky. The viscosity is similar to putty although this varies with the ratio and specific solvent used in the precipitation. Before application, the viscosity was adjusted. Usually mixing with equal parts of water, provided a readily spreadable adhesive. For some applications, one part of adhesive was mixed with up to 3 parts of water.

The adhesive can be dried to a fluffy, white powder by either freeze drying or simple evaporation. It can be reconstituted with water to the desired viscosity.

Chemical Analysis and Molecular Weight

Molecular weight analysis was done by size exclusion chromatography. Generally, molecular weights were in excess of 1 million.

Classical colorimetric analysis indicated the dried parent adhesive is 95% carbohydrate, 1-3% protein and 1% uronic acid.

Adhesion Testing

Curing

Although the adhesive sets up sufficiently for coupons to be handled within one hour at ambient temperature, full curing requires the adhesive to be well dried. Since this is a water based adhesive, drying requires extended periods of time. In an effort to decrease the drying time, a series of experiments were done where the glued coupons were incubated at increased temperatures for various time periods. The standard procedure now is to incubate glued coupons at 35 °C for 7 days. The drying chamber averages 23% humidity at this temperature.

Adhesive Reversibility

The adhesive could be cured, the cylindrical adherends pulled apart, the surface moistened, the cylindrical adherends stuck together and cured again and then pulled apart the second time with no loss in adhesive strength. The curing mechanism appears to be drying.

Resistance to Solvents

One of the more interesting properties of the adhesive was its resistance to the effects of various solvents. Cylindrical adherends were glued with the parent adhesive. Following a 10 day cure at ambient temperature, they were soaked in solvent for 48 hours, dried for 1 hour and tensile strength measured (Table II).

Table II. Effect of Solvent on Cured Adhesive

Solvent	Tensile Strength psi
Jet A fuel	932
d-limonene	922
Methylethylketone	805
Toluene	766
Control - air	728

NOTE: Tensile strength is average of 10 replicates.

Heat Stability

It was found that the microbial adhesive could withstand high temperatures, at least for a short period of time. In one experiment, after samples had been glued and cured, they were autoclaved (121 °C, 15 psi, 25 minutes). They were again allowed to cure (moisture in the autoclave disrupted the bonding), and it was found they retained the original adhesive strength (Table III).

Table III. Tensile Strength Not Decreased by Autoclaving

Sample	Tensile Strength psi
Autoclaved	811
Control (not autoclaved)	730

NOTE: Tensile strength is average of 5 replicates.

Comparison with Other Natural Polymers

There are a number of other natural polymers with adhesive properties. Some are of microbial origin, while others are derived from plants. Table IV shows a comparison of tensile strength between the microbial adhesive reported on here and other natural adhesives.

Table IV. Comparison with Other Natural Polymers

Polymer	% Solids	Tensile Strength psi
Montana Biotech parent adhesive	25	991
Cooked corn starch	25	691
Dextran	25	479
Carboxymethylcellulose	17	193
Guar gum	8	63
Xanthan gum	17	33
Sodium alginate	17	20

NOTE: Tensile strength is average of 9 or 10 replicates.

Water resistant derivatives

The parent adhesive was not particularly resistant to water and lost 80% of its strength after 30 min water immersion (Table V). A similar effect was observed after incubation in a 75% relative humidity chamber at ambient temperature.

Table V. Tensile Strength and Effect of Moisture on Parent Adhesive and Derivatives

	Dry	0.5 hr Soak	2 hr Soak	4 hr Soak	7 Days 75%
Me-A	1546	228	374	260	
Me-B	>793		30	0	138
Ac-C	874	575	324		674
Ac-D	630	247		18	289
Parent-E	809	114	22	40	
Parent-F	829				91

NOTE: Dry refers to coupons after 7 day curing at 35 °C. Soak refers to coupons being cured for 7 days, then soaked in ambient temperature water and 75% refers to coupons being cured for 7 days, then held in a 75% humidity chamber. Me A and B are 2 different methylated derivative samples, Ac C and D are 2 different acetylated derivative samples and Parent E and F are 2 different samples of the parent adhesive. Tensile strength is average of 5 replicates.

Samples with greater water resistance were prepared. The polar hydroxyl groups in the parent adhesive promote adhesion to polar surfaces like aluminum but they are also hydrophilic and lead to a low water resistance. The hydrophilicity of the adhesive may be reduced by derivatization of the hydroxyl groups with less polar groups like ethers and esters. The degree of substitution (ratio of derivatized to underivatized hydroxyl groups) can be controlled to maximize the water resistance without sacrificing adhesive strength. As expected, the derivatives produced a range of adhesive strength and water resistance and the two generally varied inversely as the degree of substitution was changed.

Substrate Effects

For shear testing, the microbial adhesive was tested on 3 different surfaces, anodized, epoxy, and CARC. Table VI shows a summary of this data.

Table VI. Shear Strength (psi) of Adhesive on Coated Aluminum Coupons

	Parent	OMe derivative	3M 4799
Anodized	819	930	171
Epoxy	576	541	146
CARC	651	533	185

NOTE: Data points are averages of 5 replicates. 3M 4799 is a commercially available adhesive included here for comparison.

Toxicology

Two toxicology tests were performed on the parent compound. It was found to be non-cytotoxic, meeting the requirements of the Agar Diffusion Test, ISO 10993. Endotoxin was found present in the preparation. Dilutions were done out to 1:256 and the corrected Endotoxin Units/ml was reported as greater than 615. By way of comparison, xanthan gum, a commercially available microbially produced polysaccharide used as a food additive, was also tested and found to have greater than 1280 Endotoxin Units/ml.

Conclusions

An adhesive was produced from a non-petrochemical feedstock by an efficient microbial fermentation. Made from a renewable resource, this water based adhesive is environmentally friendly. Its resistance to solvents makes it particularly useful in certain industrial settings. On anodized aluminum, shear strength averages 819 psi. Improved water resistance can be obtained with derivatization with ether or ester groups.

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