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A POTENTIAL BACTERIAL CARRIER FOR BIOREMEDIATION
Characterization of insoluble potato fiber

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One of the limiting factors to the effectiveness of biostimulation and bioremediation is the loss of inoculated material from the site. This can occur by a number of pathways, but is particularly problematic in open water systems where the inoculated material is simply lost in the water. It is desirable to develop new material, a matrix, within which bacteria and/or biostimulants can be incorporated.

We have investigated the basic physical properties of insoluble potato starch to eventually evaluate its use as such a matrix. Insoluble starch fibers were prepared from white potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*) and were compared for their melting temperature by DSC and their ability to bind/aggregate bacteria. The DSC curves for white and sweet potato showed that the melting temperature is 127.34 and 133.05°C for white and sweet potato fibers, respectively. The TG curves for white and sweet potato starches exhibited one main mass loss step corresponding to the DTG peak temperature at 323.39 and 346.93°C, respectively. The two types of fibers, however, showed different binding/aggregation capacities for bacteria, with white potato approximately twice as many cells of *Burkholderia cepacia* (22.6 billion/g) as cells of *Pseudomonas putida*. The reverse was true for fibers from sweet potato, binding twice as many cells of *Pseudomonas putida* (23 billion/g) as cells of *Burkholderia cepacia*.

Keywords: *bacteria, binding assay, DSC, DTG, TG, white potato (Solanum tuberosum) and sweet potato (Ipomoea batatas)*

Introduction

Bioaugmentation and biostimulation provide two alternatives in considering the bioremediation of a contaminated site [1–3]. Although distinct from each other, the two can be used as complimentary remedies. In bioaugmentation, a consortium of microorganisms is delivered to the contaminated site with the aim that members of the consortium will biodegrade the contaminant. Often however, it is found that simply adding the microorganisms is not sufficient to produce positive results. Biostimulation is then considered to aid the resident microflora in degrading the contaminant(s). However, indigenous microorganisms may actually lack the ability to breakdown that pollutant. In such a situation biostimulants alone will not have an impact [4, 5].

A problem that continues to limit the effectiveness of biodegradation is the short residence time of the exogenous microflora or the added biostimulants at the site of inoculation. This is particularly a limiting factor in bioaugmentation trials of contaminated open water systems, where inoculated microorganisms or biostimulants are simply lost in water.

A matrix within which biostimulants can be infused and/or used to aggregate bacteria for purposes of bioremediation would alleviate this problem. Immobilization of the cells on a carrier increases survival during storage, protects the cells in harsh conditions and increases persistence of the population on the site [6]. Examples of carrier materials used include sawdust, wheat bran, calcium alginate and Styrofoam [5, 7]. Ideally, such a matrix would be biodegradable, available in large supplies, can be produced with low cost, and have appropriate physical properties to allow sufficient infusion with biostimulants or aggregation of specific microorganisms of use in bioremediation. One potential candidate is the insoluble starch fibers of potato. The potential for starch to act as an aggregate material for bacteria has been demonstrated in past studies [8, 9]. Previous work, however, has focused on the using starch to extract bacteria from solutions. Such uses for aggregation of bacteria with starch have included obtaining cross sections of the microbial diversity in a location or harvesting bacteria from growth media. These experiments present promising results that encourage exploration into other applications for starch/bacteria aggregation. Little work has been done to determine if

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the aggregation potential of starch can be exploited as a carrier material for bioremediation.

Preliminary investigation in our laboratory has shown that the insoluble potato fibers, produced following extraction of soluble starch with water and water/ethanol mixtures, possess physical properties that could make it an appropriate infusion/aggregation matrix. In our studies, white potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*) were chosen as the sources of starch since they are readily available. As the test microorganisms, we use *Pseudomonas putida* and *Burkholderia cepacia*. The former has been widely used for its ability to degrade petroleum products. Experiments with *P. putida* augmented soils showed decreases in petroleum levels approximately double that of the controls [10]. Field experiments using a combination of biostimulation and bioaugmentation with *P. putida* also showed positive results at degrading petroleum [4]. Formally known as *Pseudomonas cepacia*, *B. cepacia* has been used for the degradation of phenol and trichloroethylene (TCE) [11]. TCE is one of the most important contaminants of waste sites due to widespread use and improper disposal [12]. This paper is a continuation of our previous works on the characterization of thermophysical properties of various materials ranging from biological to construction materials [13–26].

In this paper, we provide a protocol for the preparation of insoluble potato fibers and present some of their thermo-physical properties, as well as their ability to bind *P. putida* and *B. cepacia*.

Experimental

Materials

Isolating insoluble potato starch

White potatoes (*Solanum tuberosum*) and sweet potatoes (*Ipomoea batatas*) were purchased locally. The potatoes were washed, peeled, chopped and weighed. A juicer was used to separate the fiber from the juice. The fiber portion was collected and mixed with water. Soluble starch was removed by transferring the suspension to a screen and mixing under running water until the flow through ran clear. The goal was to remove as much soluble starch as possible since soluble starch has been shown to inhibit bacterial attachment [27]. The remaining fiber was soaked in 50% ethanol for several days at 4°C. Ethanol was subsequently washed out with water and excess water removed by squeezing the fiber against the screen. The fiber was frozen at –86°C overnight and then moved to the freeze drier. Further drying was carried out in an oven at 80°C for 8 h. The dried fiber was weighed and then ground into smaller pieces. To get uniform sized pieces the fiber was passed through a larger screen onto a smaller screen. Only particles that remained on the second screen were used.

Methods

Differential scanning calorimetry

Samples of dried starch particles for both white potato and sweet potato were subjected to DSC analysis using standard protocols. Sample temperature was increased from 0 to 400°C at a rate of ten degrees per minute. Approximately 4 mg of each starch type was analyzed using a Q1000 Differential Scanning Calorimeter (TA Instruments).

TG and DTG measurements

Thermogravimetric analysis (TG) and derivative thermogravimetry (DTG) on powder samples (~10 mg) were carried out using a TG Q500 T.A.I. instrument at 10°C min^{-1} from room temperature (*rT*) to 700°C under nitrogen atmosphere using a flowing rate 60 mL min^{-1}.

Binding assay

Binding of bacteria to starch particles was carried out using a modified version of the protocols described previously [8, 9, 28]. Bacterial cultures were grown overnight in LB media. A sample of the stock solution was measured for $A_{600\ nm}$ and the stock solution was then diluted to an initial concentration of 10 billion cells/mL. For each condition, 4 mL of the diluted solution was added to a plastic 15 mL sterile test tube. Each condition was performed in triplicate. From the 15 mL tube, 1 mL was transferred to a 1.5 mL disposable cuvette and $A_{600\ nm}$ was measured for each sample. This provided a starting concentration for each sample. The remaining 3 mL of cell suspension in the 15 mL tube was used for the binding assay. Each tube received 80 mg of either white potato starch or sweet potato starch. The tubes were mixed and incubated at 37°C for 1 h. After incubation, 1 mL of cell suspension was removed, without disturbing the starch particles, and measured for $A_{600\ nm}$. The binding assay was carried out for both species of bacteria with both types of starch.

The following formula was used to determine final cell concentration:

$$C_f=(A_f–A_{ns}–A_{nc})/m$$

where A_{ns} is the absorbance for the no-starch control, A_{nc} is the absorbance for the no-cells control and A_f is the final absorbance of the sample. The conversion of absorbance into cell concentration was done by divid-

ing the $A_{600\,nm}$ by the slope, m, of the appropriate standard curve.

Attachment efficiency was defined as:

$$[1–(C_f/C_i)]\cdot 100$$

where C_i is the initial cell concentration and C_f is the final cell concentration.

Statistical tests

Un-paired t-tests were used to determine if the differences in binding capacity between starches were significant.

Results and discussion

Isolation of insoluble starch fibers from whole potatoes resulted in a higher percent yield from the sweet potato than from the white potato. Insoluble fiber yields for white and sweet potatoes were 1.05 and 1.53%, respectively. Determination of percent yield was done by comparing the mass of the pealed potatoes, before isolation, to the dried starch remaining after isolation. During isolation it was observed that the white potatoes contained much more moisture than the sweet potatoes whereas the sweet potatoes were very dry and dense. The orange color of the sweet potato remained in the final product but as a faded pink/orange tint. The particles ranged from approximately 1–3 mm in size and were irregular in shape (Fig. 1).

Heating curves produced by differential scanning calorimetry (DSC) showed subtle differences in the thermal properties of each starch type. The melting peak temperature for both starches are very close (127.34 for white potato and 133.05°C for sweet potato). The melting peak for the sweet potato was broad when compared to the white potato (Figs 2 and 3); consequently more energy input per gram was required to melt the sweet potato starch. These properties, along with the observation that the sweet potato starch particles retained their pink/orange tint, suggest that the sweet potato starch was less pure than the starch of the white potato.

Fig. 1 Microscopic examination of prepared insoluble potato starch fibers (magnification: ×30). Scale divisions are 1 mm apart. Note the irregular and porous nature of the surfaces of particles

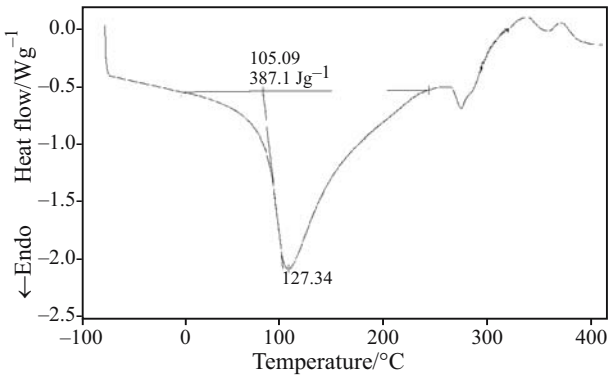

Fig. 2 DSC heating curve for white potato (*S. tuberosum*) insoluble starch

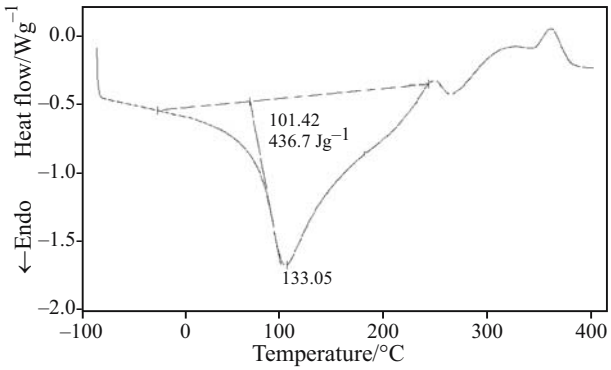

Fig. 3 DSC heating curve for sweet potato (*I. batatas*) insoluble starch

The TG and DTG curves of white potato are given in Fig. 4. The TG curve exhibits the main mass loss step between *rT*–700°C, accompanied by 79.64% mass loss corresponding to decomposition of white potato. The DTG curve showed the corresponding

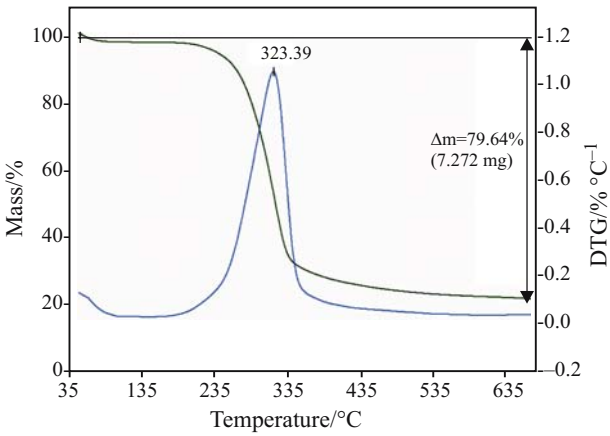

Fig. 4 TG and DTG curves for white potato (*S. tuberosum*) insoluble starch

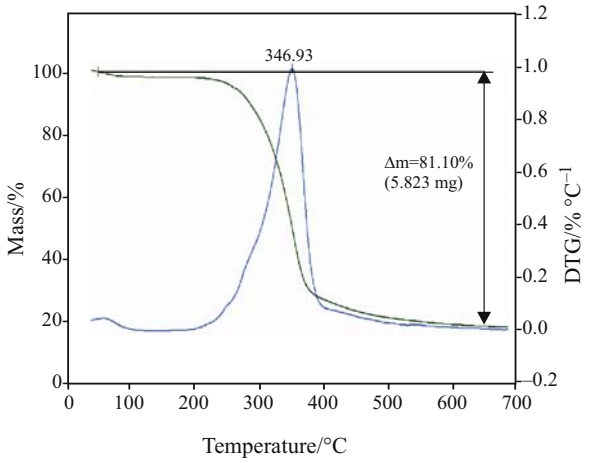

Fig. 5 TG and DTG curves for sweet potato (*I. batatas*) insoluble starch

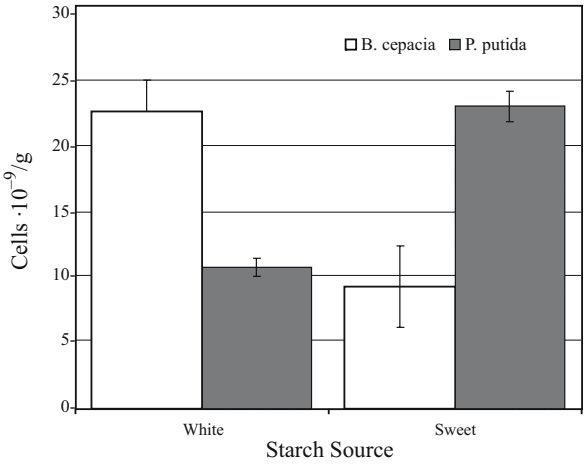

Fig. 6 Binding capacities for both white and sweet potato starch fibers showing the differences in binding between starch types and bacteria. Values on the Y axis are in billions of cells attached per gram of starch

DTG peak at 323.39°C. The TG and DTG curves of sweet potato are presented in Fig. 5. The TG curve shows the main mass loss step between *rT*–700°C, accompanied by 81.10% mass loss and corresponds to the decomposition of sweet potato. The DTG curve exhibited the corresponding DTG peak at 346.93°C.

Binding assays showed that both bacterial species were able to attach to the starch particles. Attachment efficiency was determined by comparing the initial $A_{600\ nm}$ to that of the cell solution after incubation with starch particles. Absorbance values for the final cell solution had actually increased over those of the initial cell solution. After applying the appropriate controls to the final absorbance, however, the overall absorbance decreased following incubation of cells with starch. This decrease in final absorbance, and thus final cell concentration, can be attributed to cells becoming immobilized on the surface of the starch particles. Additionally, since the surfaces of the fiber particles are irregular and porous, bacteria may be 'aggregated' within these surfaces. Average attachment efficiencies for *P. putida* were 8.9% for white potato and 20.0% for sweet potato starch. For *B. cepacia*, average attachment efficiencies were 19.2% for white potato and 7.9% for sweet potato starch. The final cell concentration included the corrections obtained from the no-starch and no-cells controls. Correction values were in terms of absorbance and were subtracted from the final absorbance values.

There were significant differences in binding capacity between starch types and bacterial species. Starch particles from white potato were able to bind *P. putida* significantly more than *B. cepacia* ($p<0.01$). Sweet potato starch particles, on the other hand, were able to bind *B. cepacia* significantly more than *P. putida* ($p<0.01$). Binding capacity was defined as the number of cells attached per gram of starch. For white potato starch particles, average binding capacities were $1.07\cdot10^{10}\pm3.6\%$ cells/g for *P. putida* and $2.26\cdot10^{10}\pm6\%$ cells/g for *B. cepacia*. Average binding capacities for the sweet potato starch were $2.30\cdot10^{10}\pm2.9\%$ cells/g for *P. putida* and $9.26\cdot10^{9}\pm19.2\%$ cells/g for *B. cepacia* (Fig. 6). This pattern observed for microbial aggregation was not anticipated, as there is no information indicating any significant structural differences between both types of potato fibers. It is not immediately clear why the sweet potato fiber for example has a higher avidity for *P. putida* than the white potato fiber. Naturally, the observed aggregation pattern would be attributed to the nature of the fiber itself and the nature of surface structures of the microorganism. It was pointed out that the prepared sweet potato fibers retained some residual pigments, as concluded from the faint orange/red color of the prepared fibers. It is conceivable that the presence of such pigments, which were absent from the white potato fibers, contributed to the observed preference of the sweet potato fiber to aggregate *P. putida*. Further experimentation is required before this point can be resolved.

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