Protein-Intercalated Bentonite as Nano-Reinforcement in Bloodmeal-Based Bioplastics

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ABSTRACT

Novatein Thermoplastic Protein (NTP) is a biopolymer produced by modifying bloodmeal to a thermoplastic material, but requires reinforcement to improve mechanical properties. Protein-intercalated calcium (CaBt) and sodium bentonite (NaBt) were investigated as nano-scale reinforcements in NTP. These were prepared by recovering proteins from meat processing wastewater called stickwater, thereby reducing treatment cost and intercalating protein into clay layers in a single step. Using stickwater-modified NaBt improved the composite’s tensile strength by 23\% (at 0.5 parts per hundred bloodmeal, pph\textsubscript{BM}) and Young’s modulus by 17\% compared to standard NTP. Stickwater treatment greatly improved the compatibility of bentonite with NTP compared to gelatin-treated bentonite whose resulting composites only had similar or slightly improved mechanical properties. The improved mechanical properties are parallel with other improvements observed in thermal, crystallinity and filler distribution. Potential applications include plant pots and seedling trays for plant nursery and agricultural disposable tools such as weasand clips, nails and barbs.

KEYWORDS: Bentonite, Stickwater, Bloodmeal, Nanocomposite, Biopolymer, Bioplastic.

1. INTRODUCTION

Conventional plastics are well known to accumulate and persist in the environment, leading to an increased focus on renewable and compostable materials using precursors such as plant or animal-based proteins and
carbohydrates.\cite{1, 2} Novatein Thermoplastic Protein (NTP) is a recent innovative biopolymer, developed by the University of Waikato and currently being trialled for commercialization by Aduro Biopolymers LP. It is manufactured from bloodmeal, a by-product of the meat processing industry, which contains about 90% protein, mainly denatured haemoglobin and bovine serum albumin. Bloodmeal is highly aggregated and insoluble, but can be converted to a bioplastic by adding sodium dodecyl sulphate, sodium sulphite, triethylene glycol, urea and water. These reduce protein-protein interactions and decrease the glass transition temperature so it can be extruded as a thermoplastic at relatively low temperatures.\cite{3, 4}

Mechanical properties of NTP in comparison with other bioplastics are given in Table 1. Research is currently focusing on increasing its tensile strength ($\sigma$), Young’s modulus ($E$) and toughness to broaden commercial applications. One approach is to use fillers such as clay or fibre as reinforcement.\cite{5} Good interfacial adhesion between the matrix and polymer is important for improving plastic mechanical properties, otherwise the filler will not disperse in the matrix, resulting in decreased mechanical properties. To achieve good interfacial adhesion, filler particle size should be small with a high surface area and must be compatible with the matrix.\cite{2, 6, 7}

One type of commonly available clays is bentonite which consists of layers of platelets with negatively charged surfaces that are loosely held together by calcium or sodium ions. These platelets can exfoliate in the biopolymer matrix, resulting in nano-scale particles with an extremely high aspect ratio and high surface area.\cite{8-10}

Proteins have side groups that can be charged, neutral or hydrophobic; possibly limiting matrix polymer intercalation into clay layers. To improve this, clay can be modified by substituting the sodium or calcium ions for

| Table 1. Mechanical properties of NTP in comparison to other bioplastics. |
|---------------------------|----------|----------|----------|----------|
|                          | $\sigma$ (MPa) | $E$ (GPa) | $\varepsilon$ (%) | References |
| NTP                       | 21.1 ± 1.8   | 1.48 ± 83.4 | -         | [4]       |
| Polylactic acid (PLA)     | 21-60       | 0.35-350   | 2.5-6     | [11]      |
| Linseed. oil polyurethane (PU) | -            | 3.4 ± 0.1   | 2.0 ± 0.2 | [2]       |
| Polyglycolic acid (PGA)   | 60-99.7     | 6-7        | 1.5-20    | [11]      |
| Poly-$\epsilon$-caprolactone (PCL) | 20.7-42   | 0.21-0.44  | 300-1000  | [11]      |
| Polyhydroxybutyrate (PHB) | 40          | 3.5-4      | 5-8       | [11]      |
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an organic modifier such as octadecylamine. This increases the interlayer spacing (d-spacing) between clay layers, promoting intercalation of the protein or polymer molecules between the clay platelets, potentially improving exfoliation as well as promoting matrix/clay interaction.\[6, 8, 12\]

Stickwater is a by-product of the meat rendering process and contains about 2-4% solids of which about 50-75% is protein (mainly gelatin and other meat-derived peptides), 25% fat and minerals \[13, 14\]. It typically has a high biological oxygen demand and is either treated before disposal or concentrated by evaporation and added to meat and bone meal. \[13, 15, 16\]

Previously, we have examined recovering proteins from stickwater and gelatin solutions using bentonite.\[14, 17\] About 60% of the protein in stickwater was adsorbed onto the clay with an organic content of 30-35 wt% dry weight (dwt) compared to 5-7 dwt% organic in its raw form. Clay modified in this manner could potentially be used as nano-reinforcement in protein-based plastics and is a more economical alternative to amine-modified clay.

In this paper, the effect of using stickwater and gelatin-modified calcium and sodium bentonite to form nano-composites using NTP as matrix was investigated. Material performance was assessed by examining the bio-composite’s mechanical, viscoelastic, crystallinity and morphological properties via tensile testing, dynamic mechanical analysis (DMA), X-ray diffraction (XRD) and optical, scanning (SEM) and transmission (TEM) electron microscopy.

In a broader perspective, we are looking at waste treatment and conversion of New Zealand meat rendering by-products. Bloodmeal and stickwater are chosen due to high organic content, highly abundant and cheap. By extracting organic from stickwater using bentonite and applying into bloodmeal-based bioplastic, their environmental impact (due to high BOD) can potentially be reduced whilst producing value-added products from what has been considered as waste.

2. EXPERIMENTAL

2.1 Materials

Calcium and sodium bentonite were supplied by Transform Minerals Limited, New Zealand. Dried food-grade gelatin was obtained from Davis Gelatin NZ Limited. Stickwater and bloodmeal were collected from Wallace Corporation bovine rendering facility in Waitoa, New Zealand. Sodium dodecyl sulfate (SDS) was purchased from Merck, sodium sulfite (SS) from BDH Labs and tri-ethylene glycol (TEG) from Orica Chemnet. All other chemicals were purchased from Ajax Finechem and were analytical grade.

2.2 Methods

2.2.1 Bentonite Modification

Previous work showed gelatin adsorption was greatest at pH 9 for CaBt and pH 5.2 (isoelectric point of gelatin) for NaBt; while for stickwater, pH 3 gave the best adsorption for both clays.\[19\] Gelatin solutions at 20 mg/mL were prepared by dissolving gelatin in 100 ml 0.02 M phosphate buffer. These were then adjusted to pH 9 for CaBt and pH 5.2 for NaBt adsorption. Stickwater was diluted to 2% by weight protein and adjusted to pH 3. Sodium and calcium bentonite were added at 3 g per 100 ml of solution. The resulting mixtures were mixed using magnetic stirrers for one hour before being centrifuged. The modified bentonite was recovered, oven
dried, ground using a hammer mill and stored prior to use in a desiccator at room temperature.

2.2.2. Fabrication of NTP-Bentonite Composites

NTP was manufactured by blending 100 g bloodmeal (sieved to 700 µm) with 40 g distilled water, 3 g SDS, 3 g SS, 10 g urea and 20 g TEG in a high speed mixer. For reinforced NTP composites, unmodified and modified bentonite were added at 0.25, 0.5 and 1 pphBM to the NTP mixture (Table 2). The mixtures were equilibrated in zip-lock bags and stored at 5°C overnight prior to extrusion and injection moulding.

The mixtures were extruded using a Thermo Prism TSE-16-TC twin-screw extruder, palletized using a tri-blade granulator (Castin Machinery, China) at 4 mm mesh and moulded into test specimens according to the ASTM D638-86 method using an Instron-33R-4204 tensile tester, fitted with a 5 kN load cell at a cross head speed of 5 mm.min⁻¹. A 50 mm extensometer was attached to the middle part of test specimen to measure strain. Load vs. extension data were obtained from the machine-interface software. All tensile values were machine-generated except for the modulus that was determined as a secant modulus between 0.0005 and 0.0025 strain. Toughness was taken as the area under the stress-strain graph up to the point of fracture and is more accurately referred to as energy-to-break. At least five samples were tested for each composition.

2.2.4 Viscoelastic Properties

Samples with the approximate dimensions of 12.8 x 6.5 x 3.5 mm were analyzed on a Perkin-Elmer- DMA 8000 using the single cantilever bending system at a temperature range of -60 to 150°C, 1 Hz frequency and 0.03 mm dynamic displacement. The samples were cooled using liquid nitrogen and heated at 2°C.min⁻¹. The storage modulus (E'), loss modulus (E'') and loss factor (tan δ) were software-generated. Glass transition temperature (T_g) was determined from the peak of tan δ curves.

### Table 2. Composite abbreviations with respect to filler type and dosage (e.g. NTP-CaBtRaw 0.25).

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Bentonite type</th>
<th>Surface modification</th>
<th>Filler dosage (pphBM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTP</td>
<td>CaBt Calcium</td>
<td>Raw Unmodified</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>NaBt Sodium</td>
<td>Ge Gelatin</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SW Stickwater</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 3. Operating parameters for extrusion and injection moulding (3, 12)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Extruder</th>
<th>Injection moulder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding zone (°C)</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Zone 1 (°C)</td>
<td>100</td>
<td>115</td>
</tr>
<tr>
<td>Zone 2 (°C)</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>Zone 3 (°C)</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>Zone 4/Nozzle (°C)</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Mould (°C)</td>
<td>-</td>
<td>70</td>
</tr>
<tr>
<td>Screw speed (rpm)</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>Torque % (Max. 12 Nm)</td>
<td>50-60</td>
<td></td>
</tr>
<tr>
<td>Injection pressure (bar)</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Back pressure (bar)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Residence time in mould (s)</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

D638-03 standard using a BOY-35A injection moulder. Table 3 outlines the parameters used for extrusion and injection moulding.

2.2.3 Mechanical Properties

Injection moulded specimens were conditioned at 23°C and 50% relative humidity for seven days prior to tensile testing. The tensile strength (σ), Young’s modulus (E) and elongation at break (ε) were analyzed according to the ASTM D638-86 method using an Instron-33R-4204 tensile tester, fitted with a 5 kN load cell at a cross head speed of 5 mm.min⁻¹. A 50 mm extensometer was attached to the middle part of test specimen to measure strain. Load vs. extension data were obtained from the machine-interface software. All tensile values were machine-generated except for the modulus that was determined as a secant modulus between 0.0005 and 0.0025 strain. Toughness was taken as the area under the stress-strain graph up to the point of fracture and is more accurately referred to as energy-to-break. At least five samples were tested for each composition.
2.2.5 Crystallinity and Clay Interlayer Spacing

X-ray diffraction (XRD) was carried out using a Philips-X'Pert X-ray machine on powdered samples at low angle configuration of $2\theta = 2^\circ$ to $40^\circ$, a scanning rate of $2\theta = 2^\circ \cdot \text{min}^{-1}$, operating at a current of 40 mA, a voltage of 40 kV using CuK$_\alpha$ radiation and a wavelength of 1.54Å. Clay interlayer spacing was calculated using the Bragg's law, $n\lambda = 2 \sin \theta$; where $n$ is the order of diffraction, $\lambda$ is the wavelength of x-ray beam, $d$ is the $d$-spacing (Å) and $\theta$ is the angle of incidence ($^\circ$).

2.2.6 Fracture Surfaces

The fracture surfaces of tensile specimens were examined using a Heerbrugg Wild 38 microscope with a Nikon DS 5MC digital sight camera attached. For SEM, samples were platinum-coated with a Hitachi E-1030 ion sputter coater and images of the surfaces obtained using a Hitachi S-4700 field emission SEM operated at 5 to 20 kV.

2.2.7 Filler Distribution

Selected unused tensile specimens were microtomed using a Reichert-Jung Ultracut microtome to less than 100 nm thickness with a diamond knife at room temperature. A colour interference card was used to determine the true section thickness after sectioning. The cut sections were carefully transferred from a water bath onto standard 200-mesh copper grids. The access water on the grids was wicked off with a wedge of filter paper and the grids were kept in a petri dish and stored at room temperature for further drying prior to analysis. Images were taken using a Philips-CM 30 TEM at an accelerating voltage of 100 kV.

3. RESULTS AND DISCUSSION

3.1 General Observations

Visual inspection of the extrudates and tensile specimens showed that the filler was well dispersed through NTP; no agglomeration of filler was noted, and tensile specimens were identical in appearance and surface finish to conventional NTP. Extrusion and injection moulding were easier using modified bentonite compared to unmodified bentonite at increasing dosage (from 0.25 to 1 pph$_{BM}$) where higher torque, back pressures and lower mass flow rates were observed. Similar observations were reported in other research.\[7, 18,19\]

3.2 Mechanical Properties

3.2.1 Tensile Strength

From Fig. 1A, stickwater-modified bentonite improved tensile strength ($\sigma$) in all composites compared to standard NTP. The highest improvement was obtained using gelatin and stickwater-modified NaBt at 0.5 pph$_{BM}$, increasing strength about 23% from 9.34 MPa for standard NTP. This compares well with previous research that gave a 20% improvement in $\sigma$ for NTP reinforced with 2 pph$_{BM}$ octadecylamine-modified bentonite.\[12\]

NTP- CaBtSW had a lower improvement in $\sigma$ of 7% for 0.25 and 0.5 pph$_{BM}$ dosages compared to NTP-NaBtSW. Composites with untreated bentonite generally resulted in lower $\sigma$ compared to standard NTP except at 1 pph$_{BM}$ dosage for both CaBt and NaBt which resulted in a slight improvement. Gelatin-modified bentonite for most of the samples had a similar effect on composite $\sigma$ as untreated bentonite, with the exception of NaBtGe at 0.5 and 1 pph$_{BM}$.

The above results suggest that untreated bentonite, particularly NaBt has poor interfacial adhesion with NTP, likely due to the clay having an overall negative charge while the matrix (also a protein) has a mixture of positive, negative, uncharged and hydrophobic groups. Haemoglobin and bovine serum albumin which make up the majority of proteins in bloodmeal have an
isoelectric point of approximately 7 and 5 respectively. Therefore, BSA has an overall negative charge, while haemoglobin is close to an overall neutral charge depending on the pH of the mixture. In both cases, it could be expected that they might have low interaction with bentonite. Calcium bentonite composites showed comparable $\sigma$ to standard NTP, possibly due to divalent ions (Ca$^{2+}$) acting as crosslinkers between the negatively charged bentonite surface and the protein.

Based on the overall increase in $\sigma$, stickwater appears to be a better filler modifier than gelatin, suggesting greater interfacial adhesion between the filler and matrix polymer. Gelatin-treated bentonite had much greater surface coverage of protein (372 mg/g for CaBt and 405 mg/g for NaBt) compared to stickwater-treated bentonite (246 mg/g for CaBt and 234 mg/g for NaBt). In contrast to food-grade gelatin, protein from stickwater is a degraded form of gelatin and other meat derived proteins, which showed little or no gelling behaviour. Poor interaction between gelatin-modified bentonite and NTP could be as a result of stronger gelatin-gelatin interactions, despite greater $d$-spacing for these modified clays. In the case of stickwater-modified bentonite, the low intra-molecular interactions and possibly smaller molecular weight allowed for better interaction with NTP.

Modified NaBt composites generally showed better $\sigma$ than similarly treated CaBt composites due to their smaller particle size. For example, gelatin-modified NaBt has a lower average particle size (20.54 $\mu$m) than its CaBt equivalent (35.72 $\mu$m), resulting in higher surface area, greater distribution and better mixing. In addition, the Ca$^{2+}$ ions are capable of holding the platelets together more strongly than the Na$^+$ and this could have reduced platelet exfoliation in the NTP matrix.

### 3.2.2. Young’s Modulus

Stickwater-modified bentonite composites improved Young’s modulus ($E$) in all samples compared to standard NTP except for 1 pph$_{BM}$ NaBtSW (Fig. 1B). The best improvement was a 17% increase in $E$ for NTP-NaBtSW0.5. Overall, modified NaBt resulted in greater $E$ than modified CaBt. Untreated and gelatin-treated bentonite generally gave lower $E$ than standard NTP. Better performance of stickwater-treated bentonite composites can be attributed to improved compatibilisation between the filler and NTP matrix. Composites with modified NaBt performed better than modified CaBt because the smaller particle size of NaBt gave greater surface area for interfacial adhesion. The poor performance of untreated bentonite composites could be attributed to poor dispersion of filler particles in the matrix and therefore poor interfacial adhesion.

### 3.2.3 Elongation at Break

Elongation at break ($\varepsilon$) was more variable (Fig. 1C) with filler addition than $\sigma$ or $E$. For CaBt composites, $\varepsilon$ generally did not decrease over that of NTP. However at low dosage, unmodified and gelatin-modified CaBt composites shared a slight improvement. A similar trend was observed for unmodified NaBt at 0.25 pph$_{BM}$ but stickwater-modified NaBt composites always had a lower $\varepsilon$ while stickwater-modified CaBt had similar elongation at break to standard NTP.
Fig. 1. Mean values of (A) tensile strength, (B) Young’s modulus, (C) elongation at break and (D) toughness of composites. Error bars indicate standard error of the mean values.
The above observations correspond to those for strength and stiffness. Composites with enhanced interfacial adhesion (for example NTP-NaBtSW) gave better $\sigma$, $E$ due to better filler dispersion. However, these fillers may have restricted NTP polymer chain extension resulting in lower $\varepsilon$. At intermediate or low interfacial adhesion, $\varepsilon$ was either the same as standard NTP or slightly higher. The highest improvement in $\varepsilon$ was recorded for unmodified NaBt corresponding to a 23.5% increase. At 0.5 pph$_{BM}$ (~0.5 wt%), this increment is high compared to a 36% increase in PLA with 4 wt% montmorillonite and a 60% increase in PLA with 4 wt% nanotitania coated with PCL.\[22\]

3.2.4 Toughness

Toughness presented in Fig. 2D is the area under the stress-strain graph for each tensile specimen and is therefore an indicator of the energy required leading to fracture. All the

Fig. 2. (A) Storage modulus and (B) tan $\delta$ curves for composites with CaBt fillers at 0.5 pph$_{BM}$ dosage.
composites showed improved toughness over NTP except for NTP-NaBtGe0.5. Unmodified NaBt composites showed some improvement in toughness with increasing filler loading, whereas it was variable for gelatin and stickwater-modified NaBt.

**3.2.5 Viscoelastic Properties**

An example of a typical DMA thermogram is given in Fig. 2 for CaBt composites. The overall shape of the storage modulus ($E'$) and tan $\delta$ curves for CaBt composites are mostly similar with only a small shift relative to NTP. Three typical viscoelastic regions can be identified from Fig. 2A: glassy (less than 0°C), transitional (0 to 100°C) and rubbery region (above 100°C).

A sharp drop in $E'$ was observed between 40 to 80°C, associated with the glass transition temperature ($T_g$) (Fig. 2B). Storage modulus in the glassy and rubbery regions were not affected by filler reinforcement, but shifted upward in the transitional region. Similar trends were obtained for NaBt composites. The $T_g$ increased from 61.8°C for NTP to between 65-75°C for all composites, suggesting protein chain movement is being restricted by the filler particles. Stickwater-modified bentonite had the lowest increase in $T_g$ followed by gelatin-modified bentonite. Filler dosage within the range tested had little effect on $T_g$ (Fig. 3) with no overall increasing or decreasing pattern observed. High filler content should increase $E'$ and $T_g$ due to an increase in stiffness. However, the trend was not observed here because the amount of filler used in NTP was very small.

**3.2.6 Crystallinity and Clay Interlayer Spacing**

XRD analysis of the composites showed crystalline regions with characteristic peaks.
between 2θ of 5° to 35° (Fig. 4). These were very similar to standard NTP without bentonite reinforcement. The first set of peaks at around 8-10° typically represent the spacing between protein β-sheets and α-helices while the peak around 18-20° represents the spacing within the α-helical turns and β-sheets.\textsuperscript{[23]}

![XRD diffractograms of NTP composites with NaBtGe fillers.](image)

Both calcium and sodium bentonite show a distinctive peak between 6-8° (Fig. 5A), corresponding to a d-spacing between clay platelets of 13.6 Å for CaBt and 10.4 Å for NaBt. Gelatin and stickwater intercalation increased the d-spacing causing this peak to occur at a smaller angle (between 4-6°). Mixing the modified bentonite into NTP resulted in these peaks disappearing altogether for all composites (Fig. 5B, C and D), suggesting exfoliation had occurred. There also appears to be a shoulder between 3-5° with the higher bentonite concentrations that could represent a very large d-spacing. This shoulder is subtle and may need to be investigated further.

The percentage crystallinity for the composites and NTP were determined by fitting a Gaussian curve representing the amorphous halo between 15° to 35° (Fig. 6). The area under the curve (total area) and halo (amorphous) from 5° to 35° were obtained and the crystalline area was calculated by subtracting the amorphous area from the total area. Percentage crystallinity was found by dividing the crystalline area by the total area. In most cases, adding unmodified or modified
Fig. 5. XRD diffractograms of (A) Untreated, gelatin and stickwater-modified bentonite; NTP composites with (B) untreated, (C) gelatin-treated and (D) stickwater-modified bentonite at 0, 0.25, 0.5 and 1 pph dosage.
bentonite increased crystallinity in NTP (Fig. 7A and B). This suggests greater amount of impurities present in the reinforced composites. In light of producing agricultural materials such as plant pots and seedling trays, these organic impurities are advantageous as added nutrients.

3.3 Morphology

3.3.1 Fracture Surface

When fracture surfaces of NTP composite samples were examined, the appearance was consistent for all test specimens (Fig. 8). SEM images of fracture surfaces showed sharp edges consistent with brittle fracture. Optical
microscope images revealed a combination of ductile failure characterised by pull-out marks and brittle fracture with cracks propagating from the edges of the tensile specimens.\(^{[24]}\)

### 3.3.2 Filler Distribution

TEM was used to evaluate bentonite platelets exfoliation and distribution in the NTP matrix at the highest filler loading (1 pph\(_{\text{w/w}}\)) for NaBt. From Fig. 9, combination of discrete particles and stacked platelets can be observed for both unmodified and modified fillers. However, the fillers are less aggregated and more distinct platelets exist at gelatin (Fig. 9B) and stickwater (Fig. 9C) modification, supporting the XRD finding that exfoliation had occurred.

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4. CONCLUSIONS

Adding stickwater-modified NaBt to NTP greatly improved composite tensile strength and Young's modulus especially at 0.5 \( \text{pph}_{\text{BM}} \) filler loading. Stickwater treatment is significantly better to enhance bentonite compatibility with NTP compared to gelatin. XRD and TEM analysis showed much greater exfoliation in NTP for stickwater-treated sodium bentonite than gelatin-treated or unmodified. The improvements in mechanical properties generated by using 0.5 \( \text{pph}_{\text{BM}} \) stickwater-treated sodium bentonite are comparable to using octadecylamine-treated sodium bentonite at 2 \( \text{pph}_{\text{BM}} \). Using stickwater-treated bentonite has a two-fold benefit: firstly, stickwater is cheaper and better filler compatibilizer for NTP compared to gelatin and octadecylamine and secondly, it reduces protein content in stickwater by 60\%, resulting in lower BOD and subsequent treatment costs.

NTP is a promising biodegradable materials particularly in agriculture. As plant pots for example, it can be directly transferred into soil and served as fertilizer as it decomposes. Depending on application, the mechanical properties of NTP can be manipulated by filler addition such as using clay.

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