# PROTEIN EXTRACTION FROM SECONDARY SLUDGE OF PAPER MILL WASTEWATER AND ITS UTILIZATION AS A WOOD ADHESIVE

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In this study, secondary sludge (SS) from a kraft paper mill was used as a source of biomass to recover protein and investigate its potential use as a wood adhesive. The process of protein recovery involved disruption of the floc structure in alkaline medium to disintegrate and release intercellular contents into the aqueous phase followed by separation of soluble protein. Finally, the soluble protein was subjected to low pH precipitation and the pelletized sludge protein, referred to as recovered sludge protein (RSP) was tested for crude protein, moisture, and other contents. A significant process yield of 90% in terms of precipitation of soluble protein from disintegrated sludge was estimated through calorimetric studies, whereas an overall material balance confirmed a RSP yield of up to 23% based on total suspended solids of raw sludge. The RSP containing 30% crude protein was used as a wood adhesive and its adhesion performance was compared with soy protein isolate (SPI) and phenol formaldehyde (PF) resin. The testing of plywood lap joints has shown up to 41% shear strength level of RSP adhesive compared to PF. This work demonstrates the technical feasibility and potential of SS as a biomass resource to develop eco-friendly adhesives for wood composite applications.

*Keywords: Secondary sludge; Waste treatment; Solid biomass; Waste management; Wood adhesive; Land-filling; Protein recovery; Recycling*

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## INTRODUCTION

The development of wood adhesives from different bio-based feedstocks has gained momentum in recent times due to stringent government regulations in the use of synthetic adhesives. Increased public awareness towards environmental and climate change issues has forced industrial manufacturers globally in adopting novel green technologies to produce biomaterials having minimal footprint on the delicate ecosystem of our planet. It is a well known fact endorsed by UN Health Services that formaldehyde-based resins, the most common and abundantly used wood adhesives, not only emit volatile organic compounds (VOC), but are also carcinogenic in nature (WHO report 2004).

The production of pulp and paper generates a large quantity of sludge, which is the final solid biomass recovered from the wastewater treatment processes. On average a typical kraft paper mill generates about 6% of its production capacity as effluent sludge, whereas in the case of de-inking operations this figure might go as high as 24% (Abubakr et al. 1995). This huge quantity of waste biomass has consistently posed serious challenges for the paper industry, requiring extra economic resources to deal with disposal and environmental issues. However, at the same time, there exist a number of excellent biorefinery and recycling opportunities to explore the utility of paper sludge in the development of value-added bio-products (Abubakr et al. 1995; Amberg 1984; Mahmood and Elliot 2006; Lagace et al. 1998). Apart from land-filling and incineration, the high ash content de-inked sludge (DS) and fibrous primary sludge (PS) have found low-volume applications as filler in the development of medium density fibreboard (MDF) panels, cement tiles, gypsum boards, and some other plastic composite materials (Abubakr et al. 1995; Davis et al. 2003; Geng et al. 2006, 2007b). In the area of wood adhesives, paper sludge had been suggested as an organic filler in the past (Robertson 1977), but no true efforts have been made to characterize and isolate adhesion ingredients as a sole formulation for a practical wood adhesive.

The secondary waste treatment plants of paper mills use activated sludge systems, which maintain a large community of heterotrophic bacteria to degrade the organic constituents in wastewater and synthesis new cells (Kirkwood et al. 2001). The exact nature of these bacteria is very complex and depends on quality of influent; however the important genera of heterotrophic bacteria include *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Citromonas*, *Flavobacterium*, *Pseudomonas*, and *Zoogloea* (Jenkins et al. 1993).

Secondary sludge (SS), generated through biological treatment of paper mill effluent, typically consists of polysaccharides, nucleic acids, enzymes, and proteins (Jung et al. 2002). Since the bacterial cells are believed to contain about 50% proteins (Shier and Purwono 1994), the SS gives an excellent opportunity to be explored for its adhesion properties. Protein-based wood adhesives, mostly derived from food crops like soybean, were in common use about half a century ago (Liu and Li 2005). Recent efforts have been focused on finding alternative feedstocks for such adhesives that do not compete with human and animal food resources. In this scenario, paper sludge, an abundant biomass residue, is an ideal feedstock to recover protein for wood composite applications. Various protein recovery protocols based on physio-chemical techniques have been reportedly used, which essentially start with solubilization of intracellular contents of sludge into the aqueous phase by disrupting the floc structure (Onyeche et al. 2002; Navia et al. 2002; Jung et al. 2001). A significant increase in the soluble protein and decrease in total suspended solids (TSS) was observed after sludge disintegration (Zhang et al. 2007; Weemaes et al. 2000).

In this work, alkali treatment was used to disrupt the floc structure of secondary sludge solids and release the intracellular proteins into the aqueous phase. Sludge protein was precipitated out through low-pH centrifugation. The composition of SS and recovered sludge protein (RSP) was determined for crude protein, ash, carbohydrates, and lignin contents. The adhesion properties of RSP for wood bonding was accessed through lap-joint shear testing, and the results were compared with commonly used synthetic and bio-based wood adhesives.

## EXPERIMENTAL

### Materials

Activated secondary sludge of about 1.5% consistency was arranged from a paper mill located in Ontario, Canada. Regent grade alkali (sodium hydroxide) and acid (sulphuric acid) solutions were used for protein recovery. Phenol formaldehyde (PF) liquid resin, 2220-109, was arranged from Arclin Canada Inc. Soy protein isolate (SPI) powder, PRO-FAM® 974, consisting 90% protein was a gift from ADM –USA. Poplar veneer was cut into strips of 25.4X101.6mm for lap joint applications.

### Methods

*Solubilization of intracellular materials and protein recovery*

The chemical method of alkali treatment (Hwang 2008) was used to disintegrate the sludge and release the intracellular materials into aqueous phase, as shown in the schematic of Fig. 1. The pH of SS was raised to 12 by adding 1.0M NaOH while constantly stirring the sludge. The disrupted floc mass containing mostly soluble protein was separated as supernatant solution by centrifugation of disintegrated sludge at 7000 rpm for 30 minutes at 4 $^{o}$C. The soluble protein was precipitated out by lowering the pH of supernatant with 2.0 M $H_2SO_4$. Four different pH levels, 1.5, 3.0, 4.5, and 5.5, were investigated to optimize the protein recovery yield. Finally these precipitates were centrifuged at 7000 rpm for 30 minutes at 4 $^{o}$C to obtain the recovered sludge protein (RSP) in the pellet form. Part of the wet RSP was used as such for wood adhesion tests, while the rest was dried at 60 $^{o}$C for 48hrs for physical and biochemical analysis.

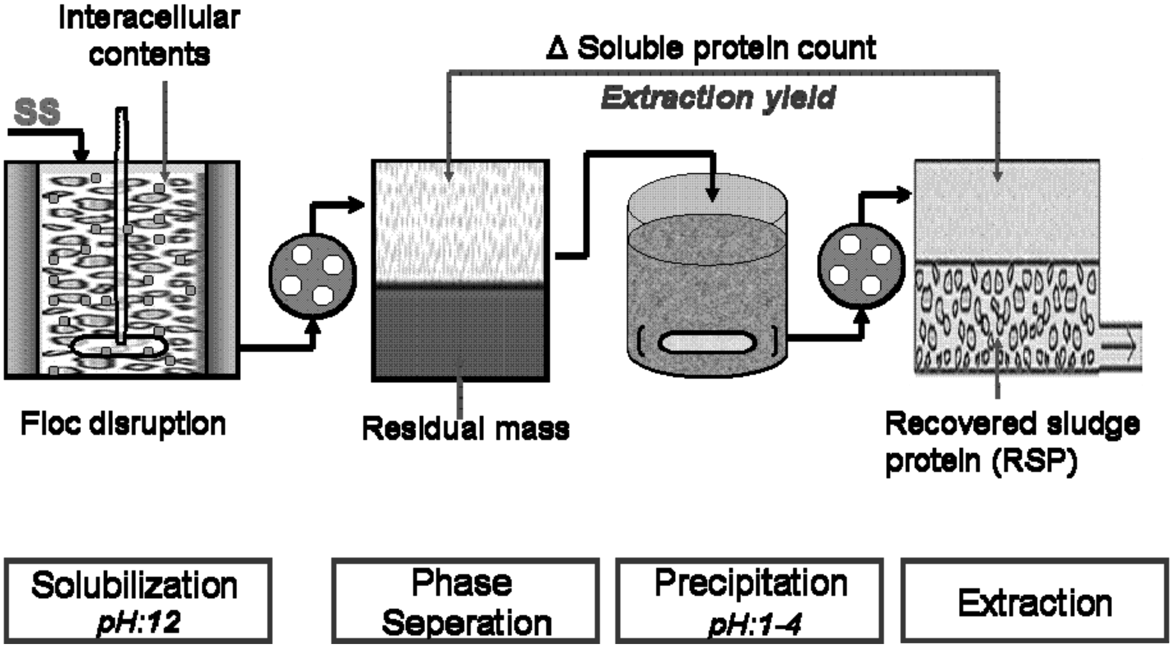

**Fig. 1.** Schematic of protein extraction from paper sludge

*Physical and bio-chemical analysis*

The solid contents, total suspended solids (TSS), and volatile suspended solids (VSS), of SS and disintegrated sludge were determined by standard methods (APHA 2005). The ash content of SS and RSP was tested by TAPPI Test Method T 211 om-07.

The soluble protein level of disintegrated sludge was measured by the Bradford method (Bradford 1976) using $\gamma$-globulin standard solution. The crude protein in SS and RSP was estimated by multiplying Kjeldahl nitrogen (John and James 1987) by 6.25, whereas total carbohydrates were determined by the anthrone method (Hedge and Hofreiter 1962), using D-glucose as the reference solution. The lipid fraction of RSP was estimated in a standard Soxhlet apparatus by using toluene as solvent and measuring the lipid content by loss in sample weight.

Klason and acid-soluble lignin contents were estimated according to TAPPI Test Method T 222 om-88. In brief, lipid-extracted sludge and RSP samples were hydrolyzed with 72% sulphuric acid for 2 h at 18 to 20 $^{o}$C, diluted to 3% acid concentration with water, and boiled for 4 h while maintaining a constant volume of the suspension. The precipitate from solution was removed by vacuum filtration, washed free of acid, and quantified for acid-insoluble lignin content. The acid-soluble lignin was determined by measuring the absorbance of filtrate at 205 nm.

*Wood composite preparation and adhesive strength*

Soybean protein isolate (SPI), containing 90% (dry basis) protein was suspended in distilled water and stirred for 2 hours to make wood adhesive of 10% solid content. The solid contents of other adhesives used are mentioned in Table 1.

**Table 1.** Summary of Wood Adhesives and their Solid Contents

| | Wood adhesives | | | |
|---|---|---|---|---|
| | PF | SPI | SS | RSP |
| Solids (%) | 49.5 | 10.0 | 15.0 | 8.4 |
| Glue Line | 5mg/cm$^2$ | | | |

PF was used as received from the supplier, whereas SS (un-treated raw sludge) was concentrated before its use as an adhesive.

Poplar veneer strips having dimensions of 10 mm x 100 mm x 3 mm were used to evaluate the bonding ability of selected adhesives. The adhesive preparations were applied on an area of 1.0 cm$^2$ to one side and one end of a poplar veneer strip (Fig. 2) in such a way that the glue line was maintained at 5 mg/cm$^2$ on a dry content basis. Adhesive coated pairs of a series of strips were stacked together and hot-pressed at 130°C for 3 min to a final combined thickness of 4.5 mm of bonded veneer pairs, which corresponded to 200 PSI pressure. After cooling at ambient conditions, the test specimens were tested for the lap-shear strength with a Zwick-z100 Testing Machine. The crosshead speed was set at 1 mm/min, and bond strength was reported as the maximum shear strength at failure of the lap-joint.

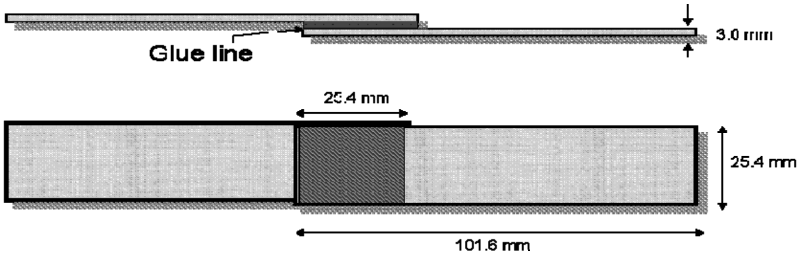

**Fig. 2.** Wood composites showing glue-line and lap-joint specimen

Water resistance of the adhesives for interior applications was evaluated by following ASTM standard method D-1183 (Standards for Wood and Adhesives), in which two consecutive cycles of heat and relative humidity (RH), (23 °C/90% RH for 60 hours and 48 °C/25% RH for 24 hours) were maintained twice in an environment-controlled chamber, Burnsco WTH-6-6-8/5. The specimens were checked for any delamination and tested for shear strength after the final cycle of weathering.

## RESULTS AND DISCUSSION

### Sludge Disintegration and Protein Recovery

The comparative values of TSS, VSS, and soluble protein concentration for untreated and supernatant of alkali treated sludge are shown in Fig. 3. As expected, with a rapid decrease in TSS and VSS, the alkali treatment at pH 12 significantly disrupted the floc cells into the aqueous phase and increased the soluble protein content by 14 times from 56 mg/L to 800 mg/L. Protein precipitation from disintegrated sludge was optimized by adjusting the pH values of centrifuged supernatant at 1.5, 3.0, 4.5, and 5.5.

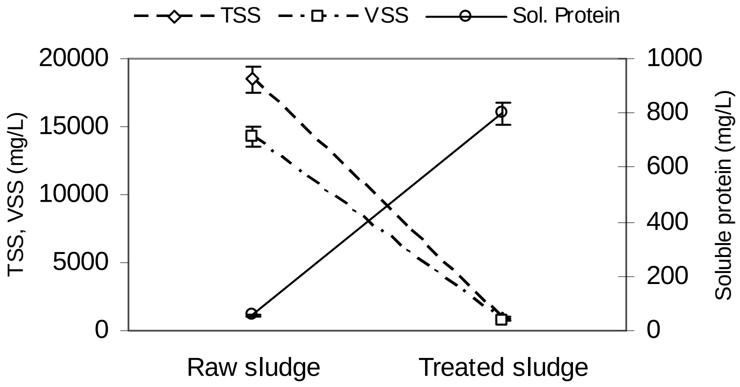

**Fig. 3.** Effects of sludge disintegration by alkali treatment on the solid contents and soluble protein concentration profiles in aqueous phase

The protein recovery efficiency was estimated by measuring the protein concentration of the supernatant after precipitation as shown in Fig. 4. Though more than 80% protein recovery was observed at pH 1.5, the maximum process efficiency was achieved at a pH value of 3.0, where 92% of the soluble protein was precipitated out. A peak protein recovery efficiency for municipal sludge has been reported by Hwang et al.

at pH value of 3.3. At higher pH values, the yield started decreasing significantly. Therefore, pH 3.0 was chosen to develop RSP samples for bio-chemical analysis and wood composites preparation.

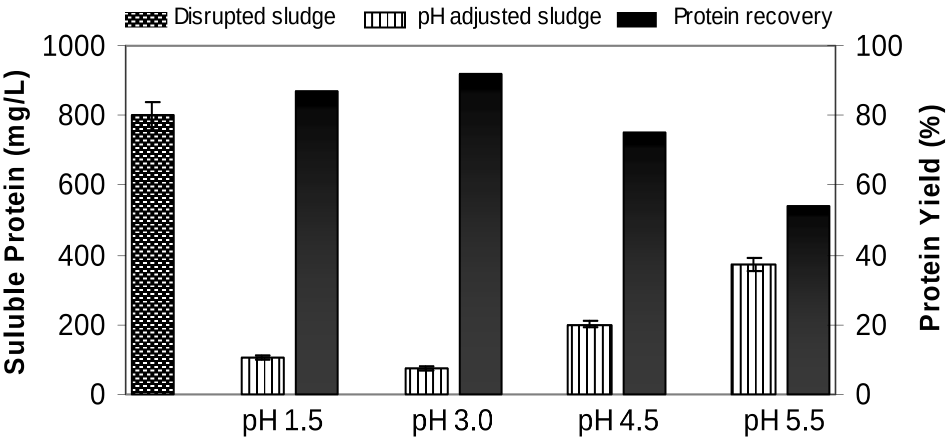

**Fig. 4.** Effect of pH on protein recovery yield

## Physical and Bio-chemical Composition

The main properties of RSP and raw sludge are listed in Table 2. A set of data taken from literature (Hwang et al. 2008) for recovered protein from municipal sludge is also given for comparison purpose. It is interesting to note that crude protein content and lipid concentration in RSP paper sludge were significantly lower than the corresponding

**Table 2.** Properties of Secondary Paper Sludge and Proteins Recovered from Paper Sludge and Municipal Excess Sludge

| | Raw paper Sludge<br>SS | Recovered protein (from paper sludge)<br>RSP | Recovered protein [a] (from municipal sludge) |
|---|---|---|---|
| Solids [b] (%) | 1.5 | 8.4 | - |
| pH | 6.7 | 3.4 | - |
| Ash | 23.3 | 13.7 | 15.4 |
| Organics [c] | 76.7 | 86.3 | 84.6 |
| Chemical composition (%) | | | |
| Crude protein | 26.8 | 33.6 | 50.1 |
| Lipids/fats | 3.7 | 0.9 | 9.0 |
| Lignin | 23.5 | 27.0 | - |
| *Klason* [d] | *20.2* | *23.3* | |
| *Acid soluble* | *3.3* | *3.7* | |
| Carbohydrate | 10.1 | 5.5 | |

a. Data for protein recovery from municipal sludge (Hwang et al. 2008)
b. Solid contents of *as received* sludge and wet cake of RSP *as recovered* precipitates
c. By difference
d. Corrected for protein and ash contents

values from municipal sludge. This is understandable, as municipal effluent is always rich in foodstuff and cooking oil residuals from household waste streams. Compared to raw sludge, the RSP contained significantly less quantity of ash and lipids, which probably relates to alkaline disintegration and subsequent phase separation steps of protein recovery. On the other hand, RSP became about 25% richer in protein content compared to raw sludge. As the ash and fat contents are believed to be detrimental for adhesion properties, their lower concentration in RSP is a positive attribute regarding wood adhesive applications. The other major nitrogen-free components in RSP are believed to be lignin and carbohydrates, which have been considered as adhesive extenders and promote adhesion properties of a wood-adhesive formulation (Geng et al. 2007a).

## Shear Strength and Failure Mode of Wood Adhesives

Dry shear strength and water resistance properties of wood composites bonded with PF, SPI, RSP and SS are shown in Fig. 5.

As anticipated, the PF (phenol formaldehyde) bonded wood composites yielded the highest strength for both dry and humidity-heat incubation studies, whereas SPI (soy protein isolate) showed better results between the two bio-based adhesives. The better performance of SPI is understandable, as it had been considerably purified, achieving a high concentration of protein compared to RSP (recovered sludge protein). Though SPI showed about 85% of the strength of PF, its water resistance strength was reduced, which is consistent with the fact that unmodified soy protein does not perform well under moist and hot conditions (Huang and Sun 2000).

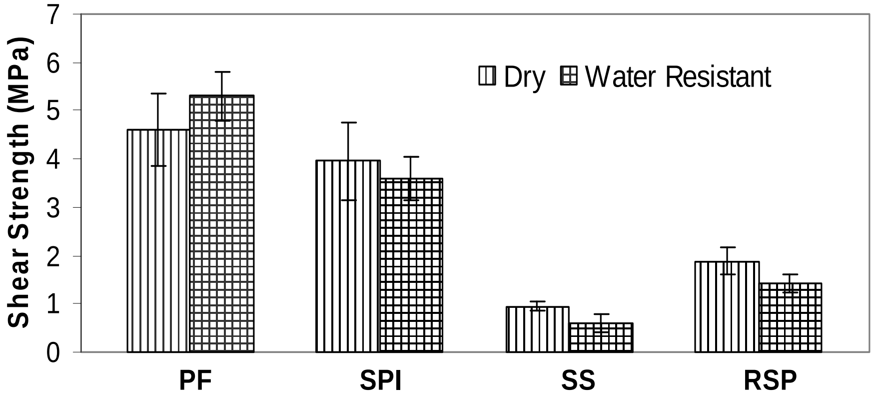

**Fig. 5.** Dry and water-resistance (*two alternate cycles each of 90%RH/23$^o$C for 60 hrs and 25%RH/48$^o$C for 24 hrs)* shear strengths of wood composites bonded with different adhesives

The RSP, a bio-based wood adhesive developed from paper sludge, showed significant improvement in bonding efficiency by yielding twice the amount of dry shear strength compared to raw sludge. Compared to PF and SPI, the dry strength of RSP was about 40% and 48%, respectively. In terms of water resistance performance, the RSP retained 75% of its strength, which was better than raw sludge, in which case 63% strength retention was observed. Though, as anticipated, PF jointed composites showed the highest retention of strength after weathering, the SPI glued lap-joints were not as efficient, because the abundant amide linkages in soy protein are hydrophilic. The other important observation regarding RSP's bonding efficiency was the study of failure mode of tested lap-joints. Though PF and SPI showed a dominant phenomenon of wood-failure, RSP also showed a mixed failure mode of both wood and substrate failure. By contrast, in the case of raw sludge, no wood failure was observed, as shown in Fig. 6.

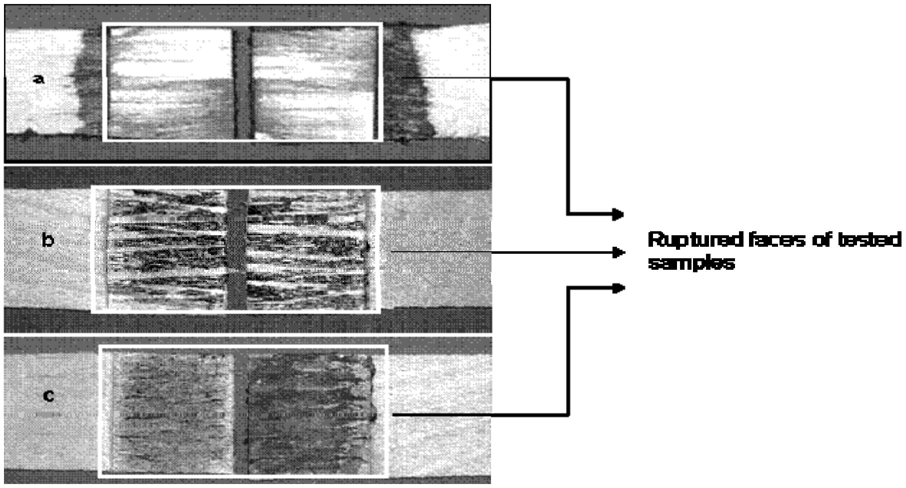

**Fig. 6.** Failure study of ruptured joint surfaces: (a) PF: total wood failure, (b) RSP: partial wood/substrate failure, (c) SS: total substrate failure

As a further study area, the improvement in adhesion strength of recovered sludge protein might involve bio-chemical modifications, such as enzymatic treatments, and purification of crude proteins to the next level. A more promising opportunity lies in the hybrid formulations of sludge proteins with other high strength adhesives such as PF that can also improve water resistant characteristics of these bio-based glues. In the long run, this work can serve as an impetus to meet the challenges of the global paper industry in terms of limited sludge disposal options by recovering value-added precursors from residual biomass.

## CONCLUSIONS

1. A high process yield was achieved to extract protein from activated sludge from a paper mill through an alkaline cell-disruptive technique.
2. The recovered sludge protein (RSP) had significantly lower ash and lipid contents compared to un-treated sludge, an interesting finding, as this is advantageous to have enhanced adhesion properties.
3. In terms of shear strength of bonded wood composites, RSP showed significant improvement as a wood adhesive compared to un-treated sludge, especially in retaining the strength after exposure to moisture and heat..
4. RSP bonded composite joints exhibited a mixed failure mode upon rupture, showing a comparable wood failure phenomenon like phenol formaldehyde and soy protein glued joints; an important feature to demonstrate bonding efficiency of wood adhesives.

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