



Bio-sludge from the Pulp and Paper Industry as Feed for Black Soldier Fly Larvae: A Study of Critical Factors for Growth and Survival

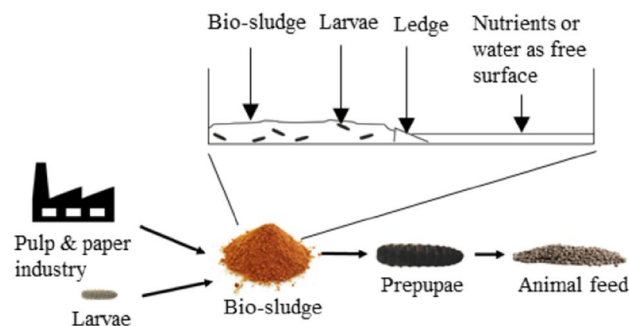
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Abstract

Pulp and paper bio-sludge (PPBS) has low economic value and is therefore often composted or incinerated. The purpose of this work is to evaluate the feasibility of using PPBS to breed black soldier fly (*Hermetia illucens*) larvae (BSFL), so improving resource efficiency and supplying protein and fat to the animal feed market. BSFL were reared on PPBS in a climate chamber on a laboratory scale in order to assess nutrient deficiency, inhibition and whether the BSFL recycle the PPBS well or not. The PPBS used came from a chemi-thermomechanical pulp/groundwood pulp mill. The effect of supplementing nutrient solution added either into the PPBS or as a free liquid surface were studied. Prepupae dry weight, survival rate until prepupae stage and residual dry PPBS were measured. The addition of reference diet leachate into the PPBS did not have a significant impact on the weight of the prepupae (2.0 mg) but the survival rate was significantly higher (16.0%). On the other hand, the addition of reference diet leachate as a free liquid surface had a significant impact on both the weight of the prepupae (4.8 mg) and the survival rate (25.7%). The bio-conversion and PPBS—reduction was as best 0.4 and 3.2% respectively. This study has proven that the nutrients in PPBS are not readily available to the larvae. Addition of reference diet leachate increase the survival rate which opens up for possibilities of co-digestion. However, it is clear that under the tested conditions, BSFL is not recycling PPBS well and BSFL as a method for recycling of PPBS need further research.

Graphic Abstract



Keywords Pulp and paper bio-sludges · Valorization · Black soldier fly larvae

Statement of Novelty

Valorization of pulp & paper bio sludge by Black Soldier fly larvae is a novel approach. Our results show that further research on nutrient availability and co-digestion are needed.

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Introduction

Treating wastewater from the pulp and paper industry produces large quantities of bio-sludge (PPBS). In Sweden alone, the yearly production amounts to 200,000–300,000 tons [1]. The composition of PPBS varies depending on the paper mill process and wastewater treatment, but typically it contains crude protein 1.5–8.3%, fat 0.3–3.3%, 10–30% dry substance (DS), lignin 17–40% DS, nitrogen

18,000–84,000 mg/kg DS, phosphorus 1,700–21,000 mg/kg DS, potassium 200–4,600 mg/kg DS and ash (27% DS) [2, 3]. It is also frequently contaminated with toxic heavy metals such as lead, mercury and cadmium [2]. Since the concentration of valuable substances is rather low, it has low economic value and is often incinerated or composted [1, 4]. From a resource conservation and circular economy perspective, there is a need to search for new and improved waste recycling technologies.

A search for recycling technologies for PPBS led to three viable recycling applications: (1) bioconversion of PPBS into protein-rich feedstock for animal fodder by single cell organisms [3] (2) bioconversion into feedstock by the black soldier fly larvae (BSFL) (*Hermetia illucens* Linnaeus; Diptera: Stratiomyidae) [2], and (3) thermo-alkaline hydrolysis of PPBS to produce second-grade cardboard and biogas [3] (Norgren et al. unpublished data). Assessment of the potential economic value of the PPBS content showed that protein generally has higher potential economic value than lignin, fat, phosphorus, nitrogen, potassium and the use of PPBS as bio fuel [2] (Norgren et al. unpublished data).

This paper investigates the potential of the BSFL as a viable recycling option to convert low-value material in PPBS to valuable protein for animal feed. The rearing of BSFL to reduce organic waste has been studied since Booram et al. [5] studied management of beef waste using BSFL. Until now, the focus has often been on converting manure or food waste into protein- and fat-rich BSFL for further processing into food and feed. Cai et al. [6] tested municipal sewage sludge as feed for BSFL and showed that BSFL can grow and survive on municipal sewage sludge. However, to the best of our knowledge no similar work has been done on PPBS. Thus, the feasibility of recycling PPBS by rearing BSFL to produce protein should be studied.

Critical factors for the prepupae weight and survival are *pathogens*: viruses, bacteria and fungi; *toxins*: heavy metals and insecticides; *nutrients*: protein, carbohydrates, fat, vitamins, and trace elements; *multiple feed sources*; *self-selection*; *ambient factors*: air temperature and humidity; and *feed properties*: pH and water content [7, 8]. In this paper, we address nutrients, *pathogens and toxins* since these are considered decisive for the design of a robust and economically viable industrial process. The impact of addition of reference diet leachate on prepupae dry weight, survival rate until prepupae stage and dry PPBS reduction were tested. PPBS from a chemical-thermo-mechanical pulp/ground wood pulp mill was used.

The purpose of this study is to evaluate the feasibility of PPBS as feed for BSFL by testing if PPBS can be digested or co-digested by the addition of extra nutrients to the sludge. BSFL were reared on PPBS on a laboratory scale.

Table 1 The design of study

Trial	Treatment	Box nr.
Control 1	Reference diet (ds 28.3%)	1–3
Control 2	PPBS (ds 20.0%)	4–6
A	PPBS (ds 20.0%) + water as free liquid surface	7–9
B	PPBS mixed with leachate (ds 20.0%) + water as free liquid surface	10–12
C	PPBS mixed with leachate (ds 20.0%)	13–15
D	PPBS (ds 20.0%) + leachate as free liquid surface	16–18

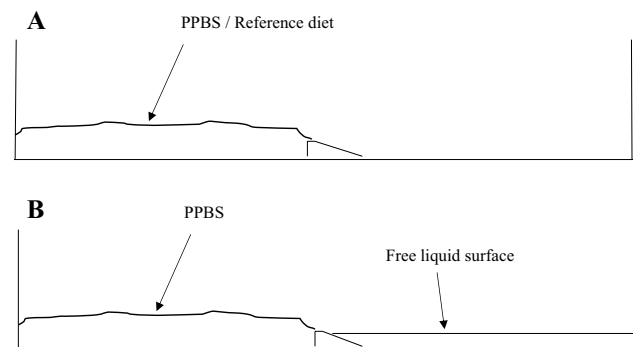


Fig. 1 Box design

Extra nutrients were added either directly to the sludge or as free liquid surface adjacent to the sludge.

Methods and Materials

Experimental Design

The experiment was designed to test the effect of supplementing nutrients and inhibiting PPBS properties by addition of reference diet leachate either mixed into the PPBS or as a free liquid surface (Table 1). Addition of water was used as a control. The experiment was carried out in (polypropylene) boxes with dimensions (21 × 17 × 15 cm). The boxes were equipped with a ledge to separate the PPBS from the free liquid surface (Fig. 1). 160 g of substrate (PPBS and reference diet respectively) was used in each trial and 100 larvae was added to each box at the start of the experiment. Evaporation was compensated for every day by addition of water. The amount of water given to the PPBS was based on the average evaporation from the PPBS only trial (control 2). Water was added to the free liquid surface to keep the weight of the box constant.

Table 2 Dry substance, pH, energy and nutrients of substrates used. Numbers within parenthesis represent standard deviation

Parameter	PPBS	Reference diet
Dry substance (%)	20.9 (\pm 0.2)	27.7 (\pm 0.1)
pH	5.2 (\pm 0.1)	4.8 (\pm 0.0)
Energy value (calc.) (MJ/kg)	3.2 (\pm 0.0)	4.5 (\pm 0.0)
Crude protein Nx6.25 (g/100 g)	1.5 (\pm 0.1)	4.0 (\pm 0.1)
Fat (g/100 g)	1.7 (\pm 0.1)	0.9 (\pm 0.1)
Carbohydrate (calc.) (g/100 g)	13.6 (\pm 0.2)	20.0 (\pm 0.0)

Materials

PPBS from a chemical-thermo-mechanical pulp/ground wood pulp mill was used in the experiments, as well as the reference diet. The reference diet was a mix of alfalfa seeds, wheat bran and maize. Table 2 shows the characteristics of the substrate used.

Preparation of PPBS

PPBS was acquired in lidded buckets from the mill. It was stored in a refrigerator until used. Before the start of the experiments 15 kg of PPBS were homogenized by hand. Mixtures of PPBS and liquid (tap water or reference diet leachate) was prepared and mixed (10 min) and the dry substance was measured. The mixtures were then put in the experiment boxes.

Preparation of Larvae

Swedish University of Agricultural Sciences delivered about 10,000 larvae, four days old in an envelope. The larvae were placed in a 21 \times 17 cm box layered at the bottom with 1 cm of wheat bran. The wheat bran was moistened with tap water to avoid dehydration and the box was stored in room temperature 1 day while the experiment was being prepared.

On the first day of the experiment, the 5-day old larvae were put in a 4.5 mm sieve. The larvae crawled through the sieve and fell onto a tray beneath where they were further separated from the wheat bran. Three times 100 larvae were counted and weighed to determine their average weight. The weight of 100 larvae was then calculated and the corresponding weight of larvae was put in each box.

Preparation of Reference Diet and Leachate

The reference diet was made from the recipe below (Fengchun 2017, personal communication):

1. 183 g of alfalfa pellets was added to 748 g of water.
2. The mix was stirred regularly by hand for 60 min to dissolve the pellets.
3. 67 g of milled maize and 75 g of wheat bran were added to the alfalfa solution.

The leachate was prepared by adding 4 L of tap water to 2.1 kg of reference diet (wet weight). The mix was then stirred by hand and stored for 24 h to settle. A syringe was used to siphon off the liquid, which was then filtered using Munktell filter paper, class II, grade 5 and put in a glass bottle with a lid. The leachate was stored in a refrigerator until use.

Climate Chamber

The climate room at More Research centre, Örnköldsvik, Sweden were used. The air temperature and humidity were set to 27.6 (\pm 0.1) °C and 63.6 (\pm 1.6) %. A 12 h day and 12 h night light scheme was used. The boxes were placed on a table at one level, ordered so that each treatment was spread horizontally to compensate for potential temperature and humidity gradient (Fig. 2).

Determination of Experimental Parameters

The following parameters were measured: Total weight of experimental boxes was measured every day (Kern PCB6000–1) and pH was measured the first day (Mettler Toledo MT225 with probe InLab Solids Pro-ISM). The initial dry substance of the PPBS and reference diet was measured based on three replicates of 20 g each which were dried in 105 °C for 24 h. All the residual feed in each box were dried for determination of the final dry substance. Air temperature and humidity in the climate chamber were measured using two standard data loggers from Clas Ohlsson, article number 36–4208–1. Final wet and dry weight of the larvae were determined using a Mettler Toledo AG204 DeltaRange. The prepupae were dried at 60 °C for 48 h. Waste reduction and bio-conversion into larvae biomass were calculated based on dry weights using Eqs. 1 and 2.

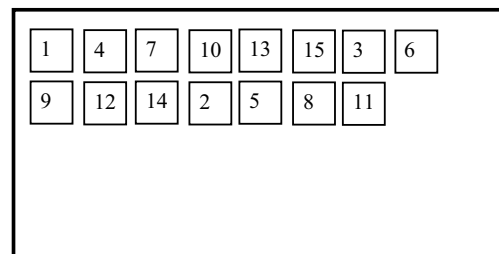


Fig. 2 Placement of boxes in the climate chamber

$$\text{Sludge reduction} = \frac{(\text{Initial weight} - \text{residual weight})}{\text{Initial weight}} \times 100\% \quad (1)$$

$$\text{Bio-conversion} = \frac{\text{Larvae biomass}}{\text{Initial sludge weight}} \times 100\% \quad (2)$$

Statistical Analysis

The results were statistically analysed with 1 factor ANOVA combined with Tukey HSD/Kramer using the Real Statistics Resource Pack software, Release 5.4 (Charles Zaiontz, www.real-statistics.com).

Results and Discussion

The dry weight of the prepupae (Fig. 3a) reared on the reference diet (control 1) was 66.5 mg, while the prepupae reared on PPBS only (control 2) weigh 0.4 mg. Addition of water as a free liquid surface (trial A) resulted in a weight of 0.6 mg and PPBS mixed with leachate combined with water as free liquid surface, 3.5 mg (trial B). Mixing leachate into the PPBS (trial C) resulted in a final weight of 2.0 mg while using leachate as a free liquid surface (trial D) had the highest prepupae weights among those reared on PPBS

(4.8 mg). Only PPBS combined with leachate as free liquid surface (trial D) differ significantly from the other treatments ($p=0.05$).

The survival rate of the prepupae (Fig. 3b) reared on reference diet (control 1) was 48.0% while the survival rate of the prepupae reared on PPBS only (control 2) was 2.0%. Addition of water as a free liquid surface had a survival rate of 1.0% (trial A) and addition of leachate into the PPBS combined with water as a free liquid surface, 10.3% (trial B). Mixing leachate into the PPBS (trial C) resulted in a survival rate of 16.0% while using leachate as a free liquid surface (trial D) had the highest survival rate among those reared on PPBS, 25.7%. Significant differences were: PPBS mixed with leachate (trial C) compared to PPBS only (control 2) and PPBS combined with leachate as free liquid surface (trial D) compared to PPBS mixed with leachate combined with water as free liquid surface (trial B).

The dry weight of the residual diet (Fig. 3c) was lower for the reference diet (control 1), 22.3 g compared to when PPBS only was used (control 2), 32.7 g. The weight of the residual PPBS when PPBS was combined with water as a free liquid surface, 33.3 g (trial A) or when leachate was mixed into the PPBS and combined with water as free liquid surface, 32.8 mg (trial B) or leachate mixed into the PPBS, 32.3 g (trial C) or leachate added as a free liquid surface,

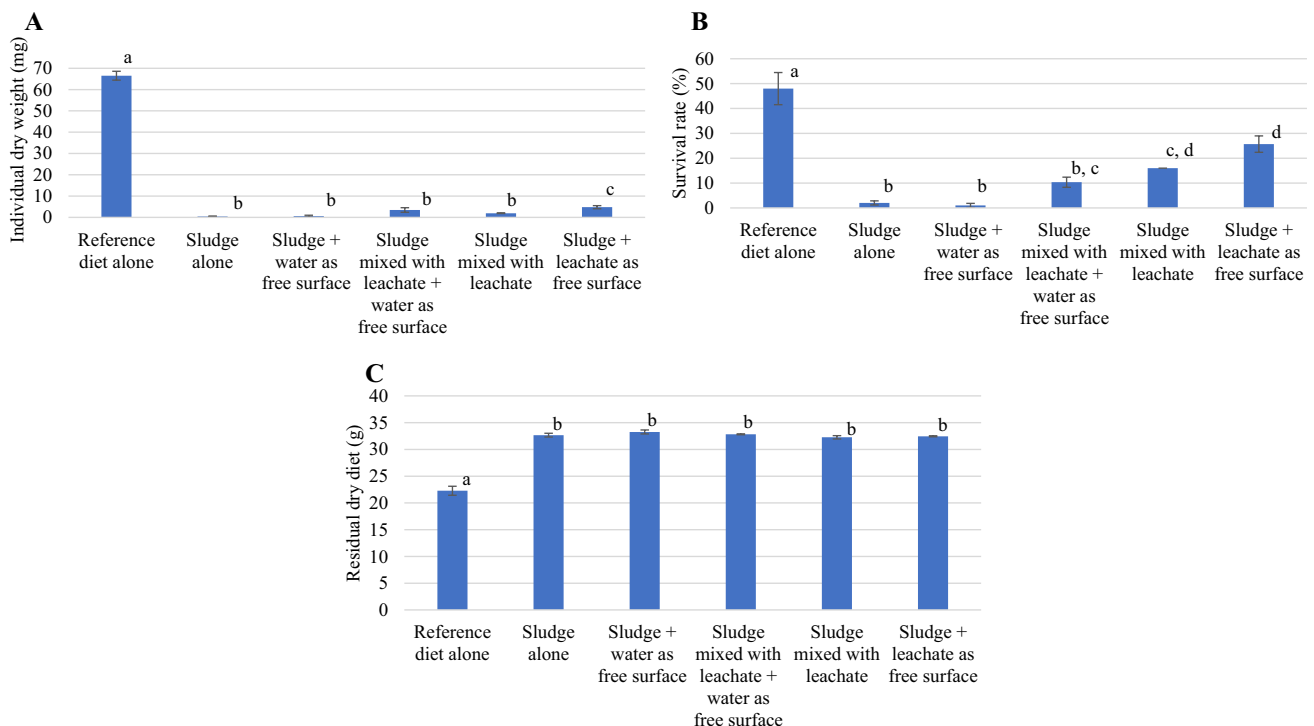


Fig. 3 Prepupae dry weight (a), survival rate (b), dry weight of residual diet (c). The error bars represent the standard deviation. The text under the bars explain the type of substrate used and liquid added. Bars marked with the same lowercase letter do not differ significantly

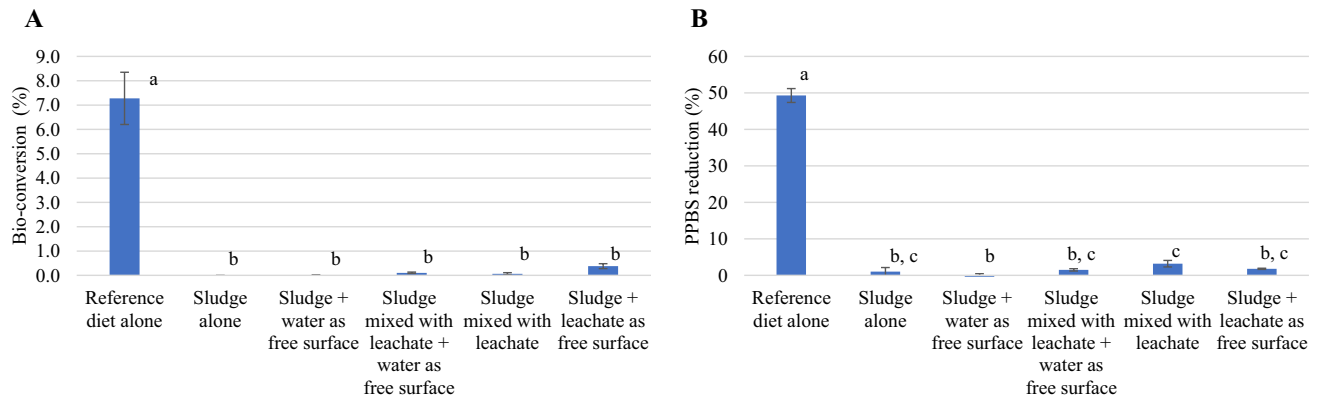


Fig. 4 Bio-conversion rate (a), PPBS reduction (b). The error bars represent the standard deviation. The text under the bars explain the type of substrate used and liquid added. Bars arked with the same letter do not differ significantly

32.5 g (trial D) did not differ significantly compared to when PPBS only was used.

The bio-conversion rate of the trial with reference diet is 7.3% (control 1) (Fig. 4a) while it is 0.0% for PPBS only (control 2), 0.0% for PPBS combined with water as free liquid surface (trial A), 0.1% for PPBS mixed with leachate combined with water as free liquid surface (trial B), 0.1% for PPBS mixed with leachate (trial C) and 0.4% for PPBS combined with leachate as free liquid surface (trial D). Only the bio-conversion of the reference diet differs significantly from the other treatments ($p=0.05$).

The PPBS reduction of the reference diet is 49.3% (control 1) (Fig. 4b) while it is 1.1% for PPBS only (control 2), 0.0% for PPBS combined with water as free liquid surface (trial A), 1.5% for PPBS mixed with leachate combined with water as free liquid surface (trial B), 3.2% for PPBS mixed with leachate (trial C) and 1.8% for PPBS combined with leachate as free liquid surface (trial D). Significant differences were: the PPBS reduction of the reference diet (control 1) compared to all the other treatments and PPBS combined with leachate as free liquid surface (trial D) compared to PPBS combined with water as free liquid surface (trial A) ($p=0.05$).

The final dry weight of the larvae receiving the PPBS was very low compared to the larvae receiving the reference diet (Fig. 3a). However, the difference in final weight is not proportional to the difference in content of nutrients of the PPBS and reference diet (Table 2), which indicates that the nutrients in PPBS are not readily available to the larvae. Lignocellulosic feed media is known to be difficult for the BSFL to utilize because of the content of crude fiber [9] where the larvae need to penetrate through the outer cuticle layer of plant products [10]. The PPBS is of plant origin and contain fibres, therefore it is possible that low availability of the PPBS nutrients contributes to the low prepupae weight.

The lack of substantial weight gain both when leachate was mixed into the sludge and administered as free liquid surface indicates that other factors than low nutrient availability such as pathogens and toxins may also be growth inhibiting to the larvae.

The fact that larvae survival rates increased significantly when leachate was added to the PPBS directly and even more when added as free liquid surface opens up possibilities for further research on how to optimize conditions for possible co-digestion of the PPBS substrate.

The inhibitive impact on larvae dry weight and survival rate could be explained if either harmful bacteria, viruses and toxins like pesticides and heavy metals or a combination of these are present in the PPBS [8]. Juvenile hormones is another a group of substances that have been proven to both impede and improve feeding of some insects [11] and found in i.e. North American native balsam fir [12].

The dry weight and survival rate for the prepupae that received PPBS was much lower than earlier published data for BSFL receiving chicken feed, chicken manure and four types food processing by-products 22.6–48.0 mg [9, 13, 14] and 72–86% [9, 14] respectively.

The low PPBS reduction (3.2%) and bio-conversion (0.4%) compared to the reference diet (49.3 and 7.3% respectively) is consistent with the results for the final larvae weight and survival rate.

Conclusion

The nutrients in PPBS are not readily available to the larvae. Simple manipulations such as adding extra nutrients (leachate) mixed into the PPBS or as free liquid surface did not significantly increase larvae weight gain or bioconversion rate but had a significant effect on larvae survival rates. This

opens up for possibilities of co-digestion of the substrate by BSFL if effects of inhibiting factors can be minimized.

Further Work

Future studies should focus on nutritional availability, co-digestion and inhibitors e.g. pathogens and toxins. Methods to pre-treat the PPBS to improve the nutritional availability and co-digestion of PPBS and feed additives should be investigated. It is also important to assess the effects of inhibitors in the PPBS and develop methods to handle these substances and organisms.

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Author Contributions All authors contributed to the study conception, design and analysis. Material preparation and data collection were performed by RN. The first draft of the manuscript was written by RN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of interest Corresponding author Robert Norgren is employed by Ragn-Sells AB.

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