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ORIGINAL ARTICLE

Original article: fermented pulp and paper bio-sludge as feed for black soldier fly larvae

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Abstract

This study evaluates the use of fermentation to increase nutrient availability in pulp and paper bio-sludge (PPBS) as feed for black soldier fly larvae (BSFL). Rearing of BSFL on fermented PPBS was carried out in a climate chamber in order to assess nutrient availability and larvae survival and growth. The PPBS used came from a chemo-thermomechanical pulp/groundwood pulp mill. The PPBS was fermented at 35 °C and 55 °C, respectively, at initial pH of 10. The effects of sediment and liquid from fermented PPBS on larvae dry weight, survival rate until the prepupae stage, bioconversion, and reduction rate of PPBS were measured. The bioconversion of the liquids (4.1–6.6%) was substantially higher than for both the sediments and untreated PPBS (≤ 0.4%). The survival rate, on the other hand, was substantially lower (26.3–30.9 %) than for the sediments and untreated PPBS (49.5–52.6%). Neither the sediments nor the liquids had significant effects on the larvae weight or on the PPBS reduction rate. The sediments had no significant effect on the survival rate or the bioconversion. This study demonstrates that fermentation dissolves a part of the PPBS and that dissolved substances in the fermentation liquid readily convert to larvae biomass. However, the bulk of the lignocellulose is not dissolved, and most of PPBS nutrients remain unavailable for growth of the larvae. Further research should focus on improved pretreatment of PPBS to increase availability of nutrients and thereby improve the feasibility of BSFL as a recycling method for PPBS.

Keywords Lignocellulose · Nutrient availability · Bioconversion

1 Introduction

Pulp and paper bio-sludge (PPBS) is produced in large quantities at pulp and paper wastewater treatment plants. It is low in nutrients and valuable substances, and it is therefore often incinerated or composted. From a resource efficiency and sustainability perspective, there is a need for other waste management options [1]. One option that has been suggested is using insects to convert this organic waste into protein- and fat-rich food and feed [2]. Producing insects as an alternative source of protein and fat has several environmental benefits compared to current livestock production. It requires less land and water, emits less greenhouse gases, and can turn organic waste into valuable food and feed [3, 4]. Black soldier fly larvae (BSFL) (*Hermetia illucens*) are considered to be the best known species for bioconversion of organic waste [3] and may be the most studied insect for this purpose [2].

The growth and development of BSFL are strongly dependent on the quality of their feed. What matters is not just the presence of nutrients but nutrient availability whether or not a nutrient is available depends on [5]:

(a) *Feed texture* such as particle size, viscosity, shear strength, plasticity, and penetration resistance
(b) *Palatability*
(c) *Intestinal mobility*
(d) *Nutrient chemical form*, that is, solid, dispersed, dissolved, or embedded in insoluble carbohydrates
(e) *The presence of antinutrients* that interfere with the absorption of the nutrient (i.e., digestive inhibitors, lectins)

Much of the current knowledge on nutrient availability is based on research on vertebrates. Unfortunately, there is limited research on the availability of nutrients for insects [5].

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Nutrient availability can be improved in several ways: reducing the content of lignin and hemicelluloses; increasing accessible surface area; reducing the crystallinity of cellulose; increasing porosity or altering lignin structure [6]; conversion of inorganic minerals into organic substances; conversion of carbohydrates, proteins, and lipids into more palatable and nutritious forms; destruction of antinutrients; increasing total sugars; denaturing protease inhibitors; sterilizing; denaturing enzymes; altering the characteristics of lipoprotein and glycoprotein [5]; and size reduction [5, 7].

Examples of technologies for improvement of nutrient availability are *physical pretreatment*, extrusion, flash sterilization [5], heat [8], shipping, grinding, milling [6, 7], irradiation by gamma rays, electron beams, and microwaves [6]; *chemical pretreatment*, alkali pretreatment [6, 8]; *acid pretreatment*, ionic liquids [6]; *physio-chemical pretreatment*, steam explosion; *hydrothermal pretreatment*, ammonia fiber explosion; *supercritical CO2 pretreatment* [6]; *biological pretreatment*, fermentation [5, 9, 10], brown/white/soft-root fungi [6, 8]; and *enzymatic pretreatment* [6], bacteria [8, 11].

PPBS consists of lignocellulose material from the pulp and paper process [12], heavy metals [13, 14], as well as bacterial cells from the wastewater treatment. 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%, dry substance (DS) 10–30%, lignin 17–40% DS, nitrogen 18,000–84,000 mg/kg DS, phosphorus 1700–21,000 mg/kg DS, potassium 200–4600 mg/kg DS, and ash (27% DS) [13, 15]. Typical values for heavy metals such as mercury are 0.045 (± 0.036) mg/kg DS, cadmium 2.2 (± 2.1) mg/kg DS, and lead 15.5 (± 25.1) mg/kg DS [13]. BSFL cannot digest lignocellulose, and thus, feed substrates with high lignocellulose content are known to impede their growth [10, 16]. Recent research on improving nutrient availability in organic waste rich in lignocellulose has focused on pretreatment by fermentation. Isibika et al. [8] tested fermentation of banana peel to improve nutrient availability to BSFL. Gao et al. [9] used four types of fermented straw to investigate the order of the most important fermentation factors affecting the fresh weight of BSFL. Pang et al. [17] used a mix of fermented pig manure and rice straw to study the effect of volatile fatty acids (VFA) on BSFL biomass accumulation. Lim et al. [18] used a mix of self-fermented CEW and soybean curd residue. Wong et al. have studied bioconversion of fermented coconut endosperm waste (CEW) in several ways: as fermented ex situ by bacteria [19, 20], as fermented in situ by yeast [21], and as fermented by fungi in situ as well as ex situ [22].

Norgren et al. [1] investigated the feasibility of using PPBS as feed for BSFL. The bioconversion rate of PPBS was low, and a possible explanation for this was the low nutrient availability caused by the high content of lignocellulose. Niero et al. (unpublished data) tested the effect of fermentation of PPBS at different pH and temperatures on solubilization of organic material and concentration of VFA. Fermentation at pH 10 gave better solubilization and higher VFA content than fermentation at a lower pH. However, to the best of our knowledge, there are no published results on the use of fermented PPBS as feed for BSFL. This article is the first in a series where we will study pretreatment of PPBS to improve its suitability as feed for BSFL. The purpose of this study is to evaluate whether bioconversion of fermented PPBS by BSFL is a feasible recycling method. We address nutrient availability and the digestion of lignocellulose and VFA since these are decisive for the design of a robust and economically viable industrial process. The impact of fermented PPBS on prepupae dry weight, survival rate until prepupae stage, bioconversion, and reduction rate of dry PPBS were tested. BSFL were reared on sediment and liquid of fermented PPBS on a laboratory scale. PPBS from a chemical-thermomechanical pulp/ground wood pulp mill was used in the experiments.

2 Methods and materials

2.1 Experimental design

The purpose was to test the effect of a diet of sediment and liquid from fermented PPBS (Table 1). The liquid was separated from the solids by centrifugation. The experiment was carried out in specially designed polypropylene boxes as previously described [1]. One hundred and twenty grams of reference diet, untreated PPBS, and sediment from centrifuged fermented PPBS were used in control 1, control 2, and trials A and B, respectively. Trials C and D used 97 g of liquid from centrifuged fermented PPBS to which 480 g of sand was added to enable stacking. Two hundred larvae were added to each box at the start of the experiment. Water was added every day to compensate for evaporation. The amount of water given was based on an ocular assessment of surface dryness. The BSFL were reared in a climate chamber.

2.2 Material

PPBS from a chemical-thermomechanical pulp/ground wood pulp mill was used in the experiments. For the PPBS content of energy, protein, fat, carbohydrate, dry weight, and pH, see earlier published characterization [1]. The PPBS content of heavy metals was previously published by Norgren et al. [13] (see mill E). The reference diet was a mix of alfalfa seeds, wheat bran, and maize; for details see Norgren et al. [1].

Table 1 Experimental design

Trial	Treatment	Box no.
Control 1	Reference diet	1–3
Control 2	Untreated PPBS	4–6
A	Sediment from fermented (35°C) and centrifuged PPBS	7–9
B	Sediment from fermented (55°C) and centrifuged PPBS	10–12
C	Liquid from fermented (35°C) and centrifuged PPBS	13–15
D	Liquid from fermented (55°C) and centrifuged PPBS	16–18

PPBS pulp and paper bio-sludge

2.3 Preparation of PPBS

PPBS was acquired from the mill and prepared for the experiments as previously described [1]. The texture of the untreated PPBS was fibrous.

Fermentation of PPBS was carried out at Luleå University of Technology. Two different fermentation temperatures were used (35 °C and 55 °C) with an initial pH of 10. Fermentation was carried out for 10 days using inoculum from anaerobic digestion of municipal sewage. A Beckman Coulter centrifuge was used with a LVA16.250 rotor at 16,000 rpm for centrifugation of the fermented PPBS for 13 min. The particle size of the fermented PPBS was fine, < 1 mm (based on ocular assessment). Samples of liquid and sediment from fermented material as well as untreated PPBS were sent to Eurofins Food and Feed testing in Lidköping, Sweden, for analysis of the glucose, fructose, saccharose, lactose, maltose, galactose, mannose, ribose, and VFA content (acetic acid, iso-valeric acid, propionic acid, iso-butyric acid, butyric acid, valeric acid, capric acid, iso-capric acid, and heptanoic acid).

2.4 Preparation of larvae

The larvae were acquired and prepared for the experiments as previously described [1] with the only exception that 200 larvae were used for each box instead of 100.

2.5 Preparation of reference diet

The reference diet was made from alfalfa pellets, milled maize, and wheat bran as previously described [1]

The particle size of the reference diet was coarse, 4-6 mm (based on ocular assessment).

2.6 Climate chamber

The boxes were reared for 16 days in a climate chamber as previously described [1].

2.7 Determination of experimental parameters

The procedure for determination of experimental parameters was described in our previous paper [1].

Reduction rate and bioconversion into prepupae biomass were calculated based on dry weights using Eqs. 1 and 2.

$$Reduction\ rate = \frac{(Initial\ weight - residual\ weight)}{Initial\ weight}\ x\ 100\% \quad (1)$$

$$Bioconversion = \frac{Prepupae\ biomass}{Initial\ sludge\ weight}\ x\ 100\% \quad (2)$$

2.8 Statistical analysis

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

3 Results

The bioconversion (Eq. 2) of liquid from PPBS fermented at 55 °C (4.1%, trial D) was substantially higher than for control 2, untreated PPBS (0.2 %). The bioconversion of liquid from PPBS fermented at 35 °C (6.6%, trial C) was even higher and close to the bioconversion of the reference diet, control 1 (8.7 %) (Fig. 1). However, none of the larvae receiving sediment, liquid, or untreated PPBS developed into prepupae (Fig. 2). Neither the sediment nor the liquid increased the dry weight of the larvae (0.7–1.0 mg and 0.7–0.8 mg, respectively) compared to untreated PPBS (0.4 mg). Furthermore, neither sediment nor liquid had higher reduction rates (Eq. 1) (2.8–6.0% and 10.6–13.0%, respectively) compared to untreated PPBS (7.8%). The survival rate of the prepupae that received liquid from PPBS fermented at 55 °C (26.3%) was lower than for the prepupae that received untreated PPBS (51.9%). The content of volatile fatty acids (VFA) in the liquid from PPBS fermented at 35 °C and 55 °C (2.0 and 2.8 g/l, respectively) was higher than that of untreated PPBS (Table 2). The content of glucose, fructose, saccharose, lactose, maltose, galactose, mannose, and ribose in sediment from PPBS fermented at both temperatures (35 °C and 55 °C) as well as in untreated PPBS was less than the detection limit (0.04 g/100 g).

4 Discussion

The higher bioconversion of the liquids (4.1–6.6%) compared to the sediments (≤ 0.4 %) shows that the dissolved nutrients in the liquids are more readily available than the non-dissolved nutrients in the sediments (although insufficient to promote larvae growth). Dumitrache et al. [23] reported that the carbohydrates in the secondary cell wall of poplar lignocellulose hydrolyze during fermentation. As the hydrolysis progresses, recalcitrant lignin appears at the surface, which eventually stops the hydrolysis, and thus, the bulk of the lignocellulose remains undigested. It is reasonable to assert that fermentation dissolves part of the PPBS, makes available nutrients that are otherwise unavailable, and thus increases digestibility. On the other hand, the bulk of the matter remains undissolved, and most of the nutrients are therefore unavailable to the larvae. Thus, a low bioconversion of the sediment occurs. The bioconversion of the liquid (4.1–6.6%) is substantially higher than that of previously reported data for municipal sewage sludge (0.2–2.3%) [24] and in the same range as coconut endosperm waste (CEW) (6%) [19] but lower than for fermented CEW (8–11.5%) [19, 21]. On the other hand, the bioconversion of the sediment (≤ 0.4%) is in the lower range of municipal sewage sludge (0.2–2.3%) [24], and the bioconversion of both the sediment and the liquid is lower than for food and feed waste (12.8–15.2%) and manure (7.1–11.3%) [24].

The concentration of VFA (Table 2) in the liquid of the fermented PPBS (1.9–2.6 g/l) increased compared to untreated PPBS (≤ 0.09 g/l). However, the weight of the larvae (0.7–0.8 mg) was as low as that of those reared on untreated PPBS (0.4 mg). Pang et al. [17] reported a substantial increase of prepupae weight at a VFA concentration of 15–26 g/l compared to 0 g/l. A possible explanation could be that the VFA concentration of the liquid used in this study is too low to affect the weight of the larvae. Furthermore, the concentration of sugars (Table 2) in the sediments of fermented PPBS (< 0.04 g/100 g) was as low as for untreated PPBS (< 0.04 g/100 g).

The individual dry weight of the larvae reared on the sediments and the liquids of fermented PPBS (≤ 1.0 mg, Fig. 1) was as low as for the larvae reared on untreated PPBS (0.4 mg) but much lower than for larvae reared on self-fermented CEW (30 mg) [18]. Similar low larvae weights were found in an earlier study on larvae reared on untreated PPBS [1]. The fact that fermentation of PPBS did not increase the weight of the larvae indicates that the fermentation applied here did not significantly increase the amount of available nutrients to a level necessary for growth of the larvae, even though some volatile fatty acids were produced. Another possible reason for the lack of growth may be that the PPBS contains substances that are growth inhibitory and that such substances still persist in sediment and liquid after fermentation.

A factor contributing to the low nutrient availability of PPBS may be the content of lignocellulose material of plant origin [25]. Lignocellulose is recalcitrant to biodegradation [26] because lignin provides protection against microbial attack and oxidation [27]. The lignocellulose is therefore largely intact after the fermentation, and the nutrients are therefore unavailable for the larvae [10, 16]. Thus, the dry weight of the larvae that received sediment or liquid from fermented PPBS was much lower than previously published weights of prepupae (22.6–48.0 mg DS) that received highly nutritious chicken feed, chicken manure, or four types of food-processing by-products [16, 28, 29]. Fermentation of PPBS as carried out in this study does not increase the PPBS reduction rate.

The fact that the larvae reared on untreated PPBS had a similar survival rate (51.9 %) to those reared on the reference

Table 2 Concentration of glucose, fructose, saccharose, lactose, maltose, galactose, mannose, and ribose and volatile fatty acids in sediment, liquid, and untreated PPBS

Material	Value
Content of each sugar in untreated PPBS (g/100g)	<0.04
Content of each sugar in sediment from PPBS fermented at 35 °C (g/100g)	<0.04
Content of each sugar in sediment from PPBS fermented at 55 °C (g/100g)	<0.04
Volatile fatty acids in untreated PPBS (g/l)	<0.09[a]
Volatile fatty acids in liquid from PPBS fermented at 35°C (g/l)	2.0±0.2[b]
Volatile fatty acids in liquid from PPBS fermented at 55°C (g/l)	2.8±0.3[c]

Numbers within parentheses represent standard deviation. Values marked with the same letter do not differ significantly. $n = 3$. *PPBS* pulp and paper biosludge

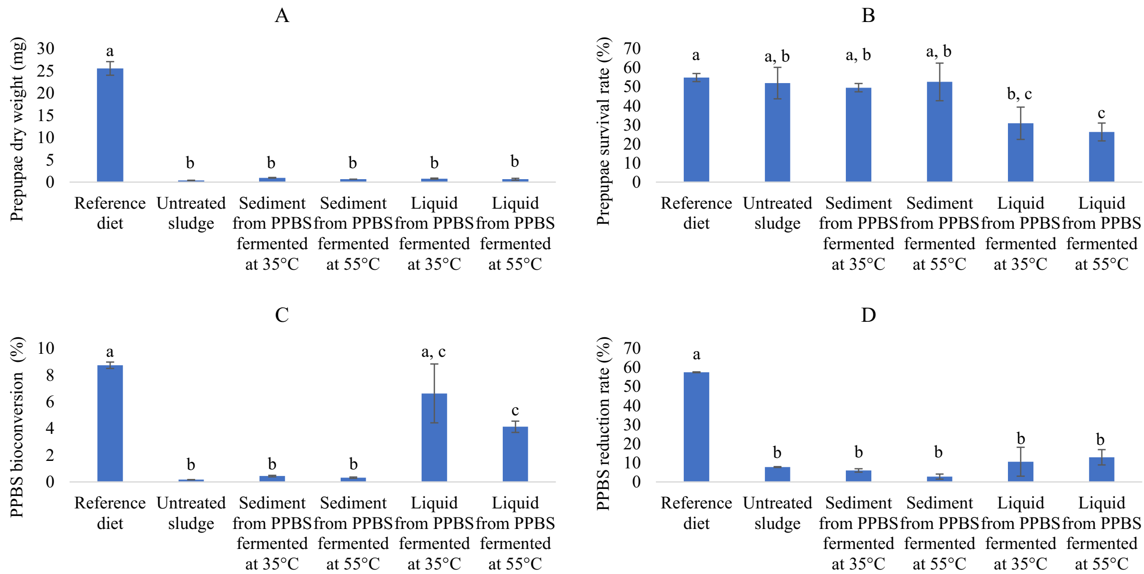

Fig. 1 Results. Prepupae dry weight (**A**), prepupae survival rate (**B**), PPBS bioconversion (**C**), and PPBS reduction rate (**D**). The error bars represent the standard deviation. The text under the bars explains the type of substrate. Bars marked with the same lowercase letter do not differ significantly. PPBS, pulp and paper bio-sludge. $n = 3$

diet (54.8 %) implies that potential pathogens and toxins present in the untreated PPBS play an insignificant role in larvae survival. The low survival rate of the larvae reared on the liquid (26.3–30.9%) may be attributed to the small amount of dry feed (0.8 g). The small amount of feed is because the bulk of the lignocellulose remains undigested [23]. Previous studies of leachate as feed for insects reported that leachate is nutritiously poor, thus causing feed shortage [30]. Thus, it is reasonable to assert that the small amount of feed in the liquid of the fermented PPBS caused the low survival rate. The survival rate of both the larvae that received liquid and sediment (26.3–52.6%) was much lower than the survival rate (72–

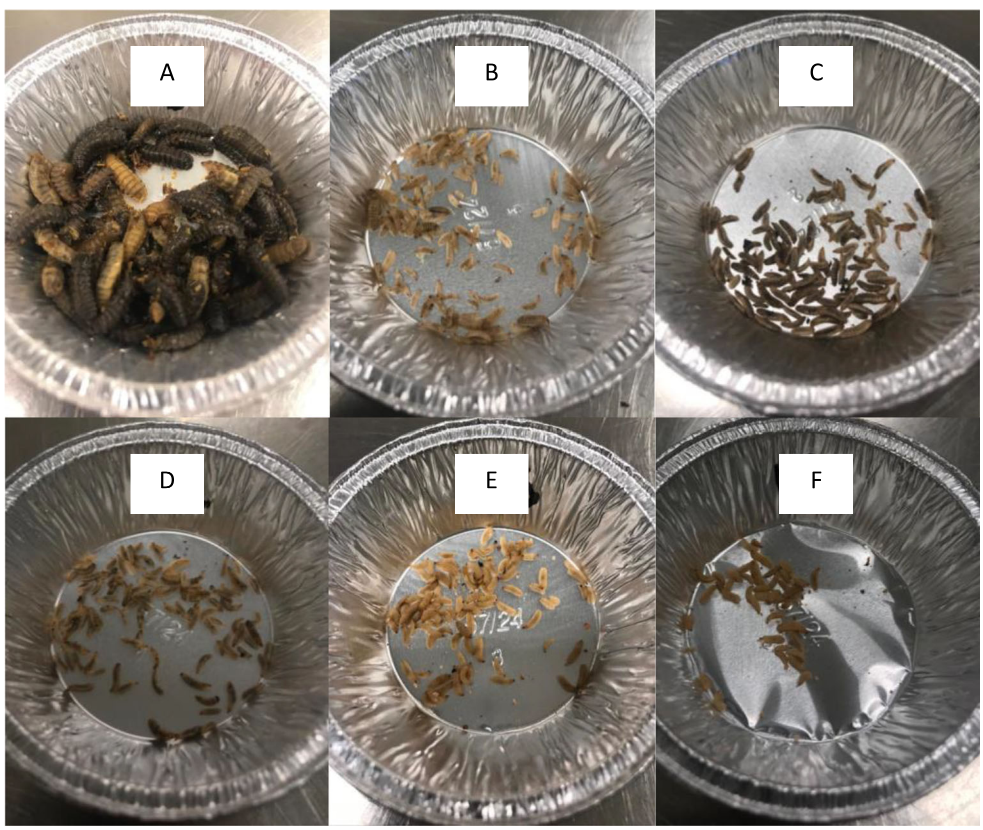

Fig. 2 Harvested larvae. Larvae reared on reference diet (**A**), untreated PPBS (**B**), sediment from PPBS fermented at 35 °C (**C**), sediment from PPBS fermented at 55 °C (**D**), liquid from PPBS fermented at 35 °C (**E**), and liquid from PPBS fermented at 55 °C (**F**)

86%) for prepupae receiving chicken feed, chicken manure, or four types of food processing by-products [16, 29]. The survival rate of 47% for the larvae receiving sediment is in line with published data for sewage sludge [31]. The result for the PPBS reduction rate (Fig. 1) is consistent with the individual dry weight and survival rate.

Another factor that may affect PPBS nutrient availability is texture [5]. Nyakeri et al. [32] observed that mixing fecal sludge with coarse textured food waste increased prepupae yield compared to mixing it with fine textured brewer's waste. The authors argue that a coarse texture facilitates larvae movement, allowing them to seek food, thereby improving prepupae yield. It is reasonable to assert that the fine texture of fermented PPBS impedes movement of the larvae and access to food, thus contributing to low nutrient availability.

The content of protein and fat in larvae have not been analyzed in this study. However, previous studies on bioconversion of fermented CEW have reported the following values: protein 15–39% [18, 20, 22] and fat 44–58% [18, 21, 22]. Analysis of protein and fat in larvae reared on PPBS is recommended for future studies.

The final weight of the larvae and the PPBS reduction rate are both low. However, a minor part of the PPBS is digested during fermentation, and those nutrients are readily available in the fermentation liquid. The bioconversion of the liquid is therefore substantially higher than for the sediment, which illustrates the need for further research on improved fermentation of PPBS. Important fermentation factors not tested in this study are water content [9, 18–21], process duration [9, 19, 20], and co-fermentation [33] as well as inoculum concentration [19–22]. Several methods to improve fermentation of lignocellulose by pre-processing have been reported: *physical* using microwaves, ultrasound, steam explosion, or heating; *biological* using microorganisms or fungi (i.e., white root fungi); and *chemical* using strong acids, alkalis, organic solvents, or ionic liquids [34].

Further research should focus on methods to increase degradation of lignocellulose by improving the fermentation process including pre-processing using physical, biological, and chemical methods [34]. Combinations of pre-processing and fermentation of PPBS should be assessed with respect to nutrient availability. Methods to improve the texture of fermented PPBS need further attention.

The cause of the high bioconversion of the fermentation liquid should be investigated. The characteristics of lignocellulose metabolites such as palatability and anti-nutritive effects may be part of the explanation and should be assessed [5]. Substances that affect palatability include rutin, sinigrin, gossypol, gamma amino butyric acid, waxes and plant secondary compounds, sucrose, ascorbic acid, sugar, amino acids, vitamin C, Mg, and K [5]. Examples of antinutrients are protease inhibitors, lectins, phytic acid, reactive oxygen species, trypsin and chymotrypsin inhibitors and other noxious-tasting substances [5]. The presence of these substances in fermented PPBS and their effect on nutrient availability should be assessed.

5 Conclusion

Fermentation dissolves a part of the PPBS, and dissolved substances in the fermentation liquid are readily converted into larvae biomass. However, the bulk of the lignocellulose is not dissolved; thus, most of PPBS nutrients remain unavailable for growth of the larvae. The BSFL did not grow well, and pretreatment of PPBS by fermentation did not increase their weight or PPBS reduction rate. This indicates a need for further research on improved pretreatment of PPBS to increase availability of nutrients and thereby improve the feasibility of BSFL as a recycling method for PPBS.

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Author contribution All authors contributed to the study's conception, design, and analysis. Material preparation and data collection were performed by Robert Norgren. The first draft of the manuscript was written by Robert Norgren. All authors commented on drafts of the manuscript and read and approved the final manuscript.

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Data Availability Not applicable

Code availability Not applicable

Declarations

Conflict of interest We have read the journal's policy, and the authors of this manuscript have the following competing interests: corresponding author Robert Norgren is employed by Ragn-Sells Treatment and Detox AB.

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