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RESEARCH ARTICLE

EFFECT OF AGRO PROCESSING WASTES ON THE PRODUCTION AND CHARACTERISTICS OF THE BLACK SOLDIER FLY *HERMETIA ILLUCENS* L.

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ABSTRACT

Background: Insects such as black soldier fly, *Hermetia illucens* are being considered as a new protein source for animal feed. Integrated development of innovative technologies for insect production using locally available agro-industrial wastes is necessary at present. **Objective**: To investigate the use of locally available agro-processing wastes namely palm kernel cake, maize bran, sorghum spent grain, soya bean waste and their combinations for maggots production. **Method**: Randomized complete blocs design with four repetitions was used. Statistical analyses were performed using SPSS Statistics 17.0 software. ANOVA was used to compare different parameters means and significant differences between the various treatments were set at $P<0.05$. **Results**: From 10 to 14 days after incubation, the percent length gained by the larvae varied from 15.34 % (palm kernel cake) to 35.2% (maize bran). There were significant differences in total weight and substrate productivity among the treatments. At 14 days after incubation, the average production of black soldier fly larvae from simple substrates varied from 152.25g (sorghum spent grain) to 446.87g (palm kernel cake). Conversion rate of the substrates to dry matter varied from 8.8% (sorghum spent grain) to 27.01% (soya beans waste). **Conclusion**: It can be concluded that soya bean waste, palm kernel cake and a mixture of the latter and maize bran could be the best substrates for producing *H. illucens* maggot for small scale producers.

INTRODUCTION

Insects are the most abundant and most diverse organisms accounting for more 70% of all species (Scaraffia and Miesfeld, 2012). Numerous crops rely on them for pollination, and their importance extends into other agricultural and human health issues (Dzerefos and Witkowski, 2014; Ingram *et al.,* 1996). Although mainly recognized as pests or nuisances affecting human, plant and animal health, insects contribute significantly to vital ecological functions providing essential ecosystem services such as pollination, waste degradation and biological control. In a recent review, Van Huis (2013) outlined the important role of insects in assuring food and feed security. Currently, insects are being considered as a new protein source for animal feed (Premalatha *et al.,* 2011). Many species of insects have been considered for their possible use in feeds for livestock and some studies have been carried out on poultry (Ravindran and Blair, 1993; Wang *et al.,* 2005; Ojewola *et al.,* 2005; Oyegoke *et al.,* 2006; Attivi, 2017), fish (Gasco *et al.,* 2014a and b) and other species (St-Hilaire *et al.,* 2007; Ng *et al.,* 2001).

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Among the different insect species, yellow mealworms (*Tenebrio molitor L.*) and black soldier flies (*Hermetia illucens*) seem to be very interesting (Schiavone *et al.,* 2014; Bovera *et al.,* 2015). They can play dual roles of recycling of organic by-products into high quality compost-fertilizers as well as utilization of the maggots directly as animal feed (Čičková *et al.,* 2012). In many parts of the world, *e.g.* Asia, several insect species have been used as complementary food sources, in particular for poultry (Ravindran and Blair, 1993; van Huis *et al.* 2013). In West Africa, when available, several insect species such as termites are opportunistically given to poultry by farmers (Ekoue and Hadzi, 2000). However, the utilization of insects as animal feeds is not yet a common practice in West Africa particularly in Togo or it is at its early stage, due probably to the lack of detailed information on available productive substrates and efficient rearing techniques of these flies. More efficient systems to mass produce maggots and implemented at farm level or feed enterprises are a paramount need or important for animal mainly poultry and fish production. Integrated development of innovative technologies for insect production using locally available agro industrial by-products and their adoption offer a high potential to globally reduce the current dependence on unsustainable soybean and fish based protein for poultry feed. However the research questions to be solved include the development of simple and effective insect production modules that can be used by small scale poultry farmers. In tropical zone, the quantitative production of *H. illucens* is possible and trivial from a large range of local resources such as kitchen wastes, agricultural by-product (vegetables and fruits) (Burton, 1973) and agro industrial by-products which could create environmental pollution if not properly managed. The objective of the current study was to test different agro-industrial by-products locally available as substrates for black soldier fly larvae production and to determine their characteristics.

MATERIALS AND METHODS

The experiment was carried out in the laboratory of the Regional centre of excellence on poultry science located in the agronomic experimentation farm of the university of Lomé 6°10 latitude north and 1°10 longitude.

Hermetia illucens rearing

Pre-pupae and pupae of black soldier flies (BSF) were obtained from the International centre of insect physiology and ecology (*icipe*) Kenya and a colony was maintained at the experimental unit of the regional Centre of excellence for poultry sciences. The rearing facility consisted of i) a large room (4m x 6m) where rectangular concrete cement bins (1m x 2m with a depth of 20 cm) on the ground were built for larvae mass production; ii) small plastic bin made by cutting longitudinally empty oil can of 25l used for the tests; iii) hatching room (3m x 4m) where eggs are incubated and adults cages (1.6m*0.8m*0.8m). The rearing followed the hereafter steps: 200 g of fermented sorghum spent grain from local brewery in plastic boxes (small basin 20 cm diameter and 2 dm^3 volume) used as ovipositing female flies attractant was introduced in the BSF adult cages made with wood and nylon mesh. On the substrate in the basin (one fourth of the tray), wooden pads were placed for egg collection. Collected eggs were incubated on palm oil cake in a tray placed in the incubation room at ambient temperature. After four days of incubation, neonates were transferred into cement bins in the lavarium where larvae were reared to pre-pupae and pupae stages. When the majority (70%) of the larvae become pupae and pre-pupae, they were then hand-collected then transferred in new containers with sawdust. When adults begin to emerge they were released in the ovipositing cages where they mate under sun light and lay eggs. One-day old eggs were collected and used in the experiments.

Substrates (agro processing wastes)

The substrates tested consisted of four agro processing by-products including palm kernel cake (PKC), soybean wastes (SW), maize bran (MB) and the sorghum spent grain (SgG). Palm kernel cake (PKC) is obtained from AVE PALM oil industry. The soybean waste is recovered from traditional processors transforming soybean into so-called soybean cheese. Maize bran is a waste from maize grain processing to fermented dough or porridge and the sorghum spent grain obtained from local sorghum based beer from traditional brewery. Simple and combinations of these substrates were tested in the current studies.

Experimental treatments and procedure

The experiments were conducted in two phases. In the first phase only simple substrates consisting of palm kernel cake (PKC), soybean waste (SW), maize bran (MB) and sorghum spent grain (SgG) were tested while in the second phase, treatments were the combinations of simple substrates (with proportion of each component on weight basis)indicated in Table 1. For each treatment, a total of 500 g of substrate was added each two days. All these combinations were based mainly on PKC but depended on the availability in quantity of the components at the time of the experiments. In both experiments completely randomized blocks design was used with four replicates. For each treatment, 250g of substrate in small plastic bin made by cutting longitudinally empty oil can of 25lwere infested with 0.1g of one day old eggs collected from the adult cage. Four to six days after incubation when newly hatched neonates are visible, for each treatment, 500g of experimental substrate were added every two days until pre-pupae appear in the substrate.

Data collected

To record the effect of each substrate on the insect growth, 100 larvae were randomly sampled in each treatment and changes in larvae length and weight were monitored every 2 days from 10 days after incubation to 14 days. At each sampling period, the selected larvae were oven dried at 65°C for 24h to determine larval dry weight. Percent dry matter was calculated using equation 1 (Eq.1):

$$\text{PDW} = \frac{LFW}{LDW} \times 100$$

where PDW = Percent dry weight; LDW = larval dry weight and LFW= larval fresh weight

To evaluate each substrate consumption, conversion rate defined as the amount of dry matter substrate converted to dry matter larvae (expressed as a percentage) was calculated using the following formula (Eq 2):

$$Conversion\ rate\ (\%) = \frac{dry\ matter\ larvae}{substrate\ dry\ matter} * 100$$

The greater the conversion rate the more efficient the substrate is at larvae.

At the end of the experiment, the quantity of dry matter of each substrate needed to produce one kg of larval dry matter was determined based on the terminology of Scriber and Slansky (1981):

$$ECD = \frac{B}{I - F}$$

where ECD = conversion efficiency of digested substrate; B = assimilated substrate used for growth (measured as larval biomass), I = total food offered during the experiment, F = residue in experimental boxes (undigested food + excretory products). High ECD values indicate high food conversion efficiency. In addition, the quantity of eggs (QE) needed to produce one kg of dried larvae was estimated with the equation below:

$$QE = \frac{\text{qe} * 1000}{LFW * PDW}$$

qe = quantity of eggs (in g); LFW = larval fresh weight (in g) and PDW = percent dry weight.

Table 1. Description of combinations of simple substrates

N°	Treatments	Description
1	PKC+SW	Mixture of palm kernel cake and soybean waste, 250g each
2	PKC+SgG	Mixture of palm kernel cake (PKC) and sorghum spent grain, 250g each
3	PKC+MB	Mixture of palm kernel cake (PKC) and maize bran, 250g each
4	SgG+MB	Mixture of sorghum spent grain and maize bran, 250g each
5	SgG+MB+ SW	Mixture of sorghum spent grain, maize bran and soya bean waste, 167g each
6	PKC+MB+SW	Mixture of palm kernel cake, maize bran and soya bean waste, 167g each
7	PKC+MB+SgG	Mixture of palm kernel cake, maize bran and sorghum spent grain, 167g each
8	PKC+SgG+SW	Mixture of palm kernel cake (PKC), sorghum spent grain and soya bean waste, 167g each
9	PKC+SW+ MB+SgG	Mixture of palm kernel cake (PKC), soya bean waste, maize bran and sorghum spent grain, 125g each

Table 2. Effect of simple substrates on maggot length (cm)

Treatments	Time (days)		
	10	12	14
	Length (cm)		
PKC	1.63±0.03 Ab	1.86±0.03 Aa	1.88±0.05 Aa
MB	1.25±0.01 Bc	1.45±0.02 Bb	1.69±0.06 Ba
SW	1.34±0.04 Bc	1.53±0.04 ABb	1.71±0.01 Ba
SgG	1.02±0.04 Cb	1.25±0.02 Ca	1.36±0.03 Ca

Means in lines with same lowercase letters and in column with same uppercase letters were not significantly different at P<0.05.
PKC=Palm kernel cake; MB = maize bran; SW = soya bean waste; SgG = sorghum spent grain

Table 3. Effect of substrate combinations on the maggot length (cm)

Treatments	Time (days)		
	10	12	14
	Maggot length (cm)		
PKC+SW	1.55±0.02 Ab	1.75±0.04 Da	1.83±0.04 Da
PKC+SgG	1.64±0.04 Aa	1.86±0.05 Ba	1.90±0.04 Ca
PKC+MB	1.51±0.04 Ab	1.83±0.08 Ca	1.85±0.09 Da
SgG+MB	1.34±0.02 Bc	1.72±0.03 Eb	1.94±0.03 Ba
SgG+MB+ SW	1.35±0.11 Bb	1.73±0.04 Da	1.94±0.06 Ba
PKC+MB+SW	1.62±0.03 Ab	1.92±0.06 Aa	2.03±0.04 Aa
PKC+MB+SgG	1.56±0.02 Ac	1.84±0.04 Cb	2.05±0.04 Aa
PKC+SgG+SW	1.55±0.02 Ac	1.79±0.03 Cb	1.96±0.04 Ba
PKC+SW+ MB+SgG	1.53±0.02 Ac	1.67±0.03 Eb	1.83±0.04 Da

Means in lines with same lowercase letters and in column with same uppercase letters were not significantly different at P<0.05.
PKC = Palm kernel cake; MB = maize bran; SW = soya bean waste; SgG = sorghum spent grain; + indicates combination of substrates

Table 4. Effect of simple substrates on maggot total weight, productivity, density and numbers at day 14

Treatment	Total maggot weight (g)	Productivity (g/kg of substrate)	Density (number/kg of substrate)	Numbers
PKC	446.87±13.87 A	137.50±4.27 A	948.54±54.29 A	3083±176,44 A
MB	345.74±10.19 B	106.38±3.14 B	897.15±64.95 A	2916±211,09 A
SW	288.98±16.79 C	88.92±5.16 C	767.69±94.01 A	2495±305,536 A
SgG	152.25±11.57 D	55.36±4.21 D	752.09±92.43 A	2068±254,187 A

Means in lines with same lowercase letters and in column with same uppercase letters were not significantly different at P<0.05.
PKC = Palm kernel cake; MB = maize bran; SW = soya bean waste; SgG = sorghum spent grain

Moreover larval density in each substrate and substrate larval productivity were determined.

Statistical analyses: Statistical analyses were performed using SPSS Statistics 17.0 software. ANOVA was used to reveal significant differences between the various treatments.

RESULTS

Effect of the substrates on BSF growth

The substrates and the sampling periods significantly affected the growth of the larvae of the BSF (Table 2). In the simple substrates, the length significantly increased with the sampling period (days after egg incubation) (P<0.05). At each sampling date, larvae reared on PKC were significantly longer than on all other treatments. The lowest larvae length was obtained with sorghum spent grain. However, from 10 to 14 days after incubation, the percent length gained by the larvae varied from 15.34 % (PKC) to 35.2% (maize bran) indicating a low growth of larvae on maize bran. Similar to the simple substrates, larvae length significantly increased over time in all combinations of the substrates and varied from 1.34 cm (mixture of sorghum spent grain and maize bran, at 10 days after incubation) to 2.05 cm (mixture of PKC, maize bran and sorghum spent grain at 14 days after incubation) (Table 3). Across sampling periods, the lowest lengths were obtained in sorghum spent grain + maize bran combination and in the mixture of sorghum spent grain + maize bran +soya bean waste while the highest lengths were observed in the mixture of PKC+ maize bran + soya bean waste and PKC + maize bran + sorghum spent grain with the growth rate varying from 15.85% (PKC + sorghum spent grain) to 44.78% (Sorghum spent grain + maize bran).

BSF larvae production

Total maggot fresh weight, substrate productivity, maggot density and numbers

Table 5. Effect of substrate combinations on maggot total weight, productivity, maggot density and numbers at day 14

Treatment	Total maggot weight (g)	Productivity (g/kg of substrate)	Density (number/kg of substrate)	Numbers
PKC+SW	288.07±28.81D	128.03±11.0D	850.00±135.26B	1912±304.34B
PKC+SgG	305.52±14.21C	135.79±6.32C	852.11±84.93B	1917±191.10B
PKC+MB	377.98±24.81A	167.99±11.03A	1124.44±137.78A	2530±310.00A
SgG+MB	247.37±14.41E	109.94±6.40E	786.67±63.54B	1770±142.97B
SgG+MB+ SW	192.94±30.37E	85.75±13.50E	468.44±108.57C	1054±244.28C
PKC+MB+SW	209.59±25.64E	93.15±11.39E	525.33±93.13C	1182±209.55C
PKC+MB+SgG	155.97±32.69E	69.32±14.53E	366.67±90.41C	825±203.43C
PKC+SgG+SW	280.66±23.63D	124.74±10.5D	788.11±121.28B	1773±272.88B
PKC+SW+ MB+SgG	318.24±8.61B	141.44±3.82B	1021.67±41.98A	2299±94.46A

Means in lines with same lowercase letters and in column with same uppercase letters were not significantly different at P<0.05.
PKC = Palm kernel cake; MB = maize bran; SW = soya bean waste; SgG = sorghum spent grain; + indicates combination of substrates

Table 6. Effect of simple substrates on maggot dry matter (%)

Treatment	Time (days)		
	10	12	14
	Maggot dry matter (%)		
PKC	30.43±0.00 Bc	34.31±0.01 Ab	37.34±0.01 Aa
MB	32.96±0.01 Ab	34.43±0.01 Ab	38.31±0.00 Aa
SW	30.66±0.00 Bb	32.98±0.01 Ab	39.48±0.02 Aa
SgG	26.23±0.00 Cb	26.30±0.01 Bb	30.18±0.00 Ba

Means in lines with same lowercase letters and in column with same uppercase letters were not significantly different at P<0.05.
PKC = Palm kernel cake; MB = maize bran; SW = soya bean waste; SgG = sorghum spent grain;

Table 7. Effect of substrate combinations on maggot dry matter (%)

Treatments	Time (days)		
	10	12	14
	Maggot dry matter (%)		
PKC+SW	27.49±0.01 Ac	36.99±0.00 Aa	32.83±0.01 Eb
PKC+SgG	27.03±0.00 Ab	34.05±0.00 Ca	31.70±0.01 Fa
PKC+MB	25.16±0.02 Ab	33.66±0.01 Dab	39.63±0.04 Aa
SgG+MB	26.90±0.02 Ab	31.74±0.00 Ea	33.43±0.01 Da
SgG+MB+ SW	24.64±0.02 Ab	34.95±0.02 Ba	36.59±0.02 Ba
PKC+MB+SW	24.21±0.01 Ab	36.92±0.00 Aa	38.15±0.02 Ba
PKC+MB+SgG	29.23±0.01 Ab	35.76±0.00 Ba	31.76±0.03 Fab
PKC+SgG+SW	26.37±0.01 Ac	34.30±0.00 Cb	36.91±0.02 Ba
PKC+SW+ MB+SgG	26.07±0.01 Ab	34.39±0.00 Ca	33.87±0.01 Ca

Means in lines with same lowercase letters and in column with same uppercase letters were not significantly different at P<0.05.
PKC = Palm kernel cake; MB = maize bran; SW = soya bean waste; SgG = sorghum spent grain; + indicates combination of substrates

Table 8. Quantity of substrate and of BSF eggs estimated to produce 1kg maggot dry matter

Treatments	Egg quantity (g)	Substrate quantity (kg)
PKC	0.60	6.81
MB	0.75	7.601
SW	0.88	3.702
SgG	2.18	11.36
PKC+SW	1.06	5.68
PKC+SgG	1.03	6.27
PKC+MB	0.67	4.95
SgG+MB	1.21	6.8
SgG+MB+ SW	1.42	6.69
PKC+MB+SW	1.25	7.41
PKC+MB+SgG	2.02	12.87
PKC+SgG+SW	0.97	4.85
PKC+SW+ MB+SgG	0.93	5.11

PKC = Palm kernel cake; MB = maize bran; SW = soya bean waste; SgG = sorghum spent grain; + indicates combination of substrates

Table 4 showed the effect of simple substrates on total larval weight, substrate productivity, larval density and number at day 14. There were significant differences in total weight and substrate productivity among the treatments. At 14 days after incubation, the average production of BSF larvae from simple substrates varied from 152.25g (sorghum spent grain) to 446.87g (PKC). The highest productivity was obtained from PKC and the lowest production from sorghum spent grain. But larval densities and numbers were not affected by the substrates tested. Combinations of simple substrates have significant effects on total weight, productivity as well as on larval density and numbers (Table 5). The highest total weight was obtained from PKC and the lowest from PKC+MB+SgG. Similar trend was observed as for productivity, larval density and numbers. Combined substrate productivities ranged from 69.32g/kg substrate (PKC+MB+SgG) to 167.99g/kg substrate (PKC+MB).

Effect of substrates on Maggot dry matter

Table 6 indicated that the percentage of dry matter in the tested substrates increased across the sampling date with the highest at 14 days. At each sampling date, the larval dry matter produced with sorghum spent grain was significantly the lowest compared to the other substrates which were statistically similar at 12 and 14 days after incubation. Conversion rate of the substrates to dry matter varied from 8.8% (sorghum spent grain) to 27.01% (soya bean waste). Similar trends were observed across sampling dates when using the simple substrates combinations (Table 7).At 14 days after incubation, the lowest rate of dry matter was obtained with the following combinations: PKC + sorghum spent grain; PKC+maize bran+sorghum spent grain and PKC+ soya bean waste. The highest maggot dry matter rate was obtained with PKC + maize bran.

Estimation of substrate and egg quantities needed to produce 1kg of maggot dry matter in the current experiment conditions

According to Table 8, less than one gram of egg and less than 5 kg of SW, PKC+MB, PKC+SW+SgG were needed to produce one kilogram of maggot dry matter. Sorghum spent grain and PKC+MB+SgG used the highest quantity of eggs (> 2g) and substrate (> 11.4kg) to produce one kg of maggot dry matter.

DISCUSSION

The quantity but also the quality of the food are important factors for the growth and development of all living organisms including insects. The results of the present study indicated that BSF larvae could be produced on different organic wastes namely PKC, sorghum spent grain, maize bran and soya bean waste and their combinations. These results are similar to the findings from Newton et *al.* (2005); St-Hilaire *et al*. (2007), Hem *et al.* (2008); Martínez-Sánchez *et al.* (2011) who demonstrated that *H. illucens* larvae could feed on diverse organic wastes, including plant debris, decaying animal and manure. In contrary, Bouafou (2011) found that plant derived wastes have less maggot productive potential. The productive capacity of a waste to produce BSF would therefore depend on the nutrient content and the putrescence capacity of the substrate. In the current experiment, the high productivity of maggots obtained from palm kernel cake is due not only to its high protein and fat content but also its texture compared to soybean waste which is richer in protein and fat but has too high moisture content that would be a hindrance to the development of BSF larvae. Therefore, it can be hypothesized that an optimal moisture content of substrate either by adding to substrates with high moisture content a more or less dry substrates or prior drying them could positively affect the production of BSF maggots. However, De Bakker *et al*. (2014) found no significant effect of water content on larval growth. More research is therefore needed to document further the effect of different moisture content of the substrates for BSF production. The average production of BSF larvae from tested organic by-products suggested that 13.8, 10.6, 8.9 and only 5.5% of the PKC, MB, SW, and sorghum spent grain, respectively were converted into larval biomass. The conversion of the simple organic substrates and the transformation rate of combined substrates varying from 8.6 (SgG+MB+SW) to 16.8% (PKC+MB) were lower than the reported value from Burtle *et al*. (2012). These authors reported that the feed conversion rates in BSF larvae production could be up to 25% (dry matter basis). Similarly, the ESR international LLC (2008), a global waste management team, noted that roughly 20% of the fresh food wastes could be converted into fresh larvae. The variation in larvae production from various wastes might be due to the differences in putrescent capacity and nutritional quality of the waste materials and the rearing conditions. Additionally, the production could be affected by the hatching rate expressed as resulted larval density. During the experiments, it is observed that PKC had the highest egg hatching rate and could be recommended as pre-incubation substrate. In the current experiment, the individual length of the larvae reared on different waste materials indicated that at day 14, the average lengths were between 13.6 mm and 18.8 mm for simple substrate and they varied from 18.3 to 20.3 mm for composite substrates. These results were below the findings of Diclaro *et al.* (2009) who reported that the BSF larvae can reach a maximum length of 27 mm in their last larval stage when they are dull and whitish in colour. The differences in the observed length of individual larvae might be due to the difference in the nutritional value of their growing media and/or the density of larvae in the bin compared to the tested substrates in the current study.

The length of the larvae could also be affected by the size of the bin used for the rearing and the number of individual larvae in the bin. All these factors could affect the overall development of the larvae. From ten days after incubation to 14 days, the percent length gained by the larvae varied from 15.34 % (PKC) to 35.2% (maize bran) indicating that there was a rapid development of larvae in PKC compared to the other substrates used. According to (Ateng *et al.,* 2016), differences in the nutrient content of feed materials can lead to different growth patterns. In fact, the highest average size was recorded on PKC which contains 14-20% proteins and 6-15% lipid while the lowest was observed on fermented sorghum spent grain with less than 14% of proteins and 8.8% lipids but with higher carbohydrates. According to Bouafou (2011), the digestive enzymes of maggots act mainly on proteins and lipids. Moreover, sorghum spent grain contains tannins (Larbier and Leclercq, 1992) which might have affected the feeding of the larvae. Other factors include the compactness (very low porosity) of sorghum by product reducing the mobility of the larvae and their aeration. Generally, during daylight or under appropriate light, the larvae go into the substrate and probably require oxygen to feed on the substrate. Aeration of the substrate by mowing it would increase the growth rates and the characteristics of the larvae. The nutrient content of the tested substrates affecting larval growth rate had consequently affected the larvae dry matter content. Fourteen days after incubation, the highest percentage of dry matter of the harvested larvae was estimated to about 39.5%; this is higher than the rate estimated by van Arnold *et al*. (2014). All of these parameters would depend on the hatching rate, which itself is a function of temperature, moisture and aeration of the substrate (Tomberlin *et al.,* 2001).

Conclusion

BSF larvae could be produced on different organic wastes namely PKC, sorghum spent grain, maize bran and soya bean waste and their combinations. Growth rate, incubation rate and the productivity are the highest on PKC. Further studies should be conducted to find appropriate density, temperature and the feeding scheme to optimize BSF production system at local and semi industrial levels.

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