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Accelerated decomposition of sugarcane crop residue using a fungal–bacterial consortium

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Abstract

A fungal–bacterial consortium provided by Sybron Chemicals, Inc. was used in a laboratory study in an effort to accelerate the decomposition of post-harvest sugarcane residue. The consortium includes the fungus *Ceriporiopsis subvermispora* and bacterium *Cellulomonas* sp., which were chosen for their cellulolytic capabilities and *Azospirillum brasilense*, which was chosen because it is a nitrogen-fixing bacterium. Study results indicated that the consortium sprayed over the residue and mixed in soil supplemented with 0.3% molasses showed a significant difference for several parameters. Those measurable differences included visual decomposition of residue, bacterial and fungal populations, soil pH, nitrogen, and available phosphorous when compared to control and the treatment in which the consortium was sprayed over the residue but not mixed. This study indicates that this fungal–bacterial consortium may accelerate the decomposition of post-harvest sugarcane residue provided the residue is mixed with soil. Further study is necessary to refine the process for the future application of this consortium as a possible alternative to the current practice of open air burning of sugarcane residue by farmers.

Keywords: Crop residue; Biodegradation; Cellulolytic microbes; Composting; Sugarcane

1. Introduction

In the sugarcane growing areas of southeast Louisiana air pollution results from burning sugarcane residue after sugarcane harvest. In Louisiana air pollution due to open air burning is estimated to be 21% of total air pollution from all sources (Parker, 1970). In 1998, 804 producers in 23 parishes grew sugarcane on 427,930 acres. An estimated 393,700 acres of sugarcane was harvested, with a total production of 1,241,994 tons of sugar. Sugar production is one of the major industries in Louisiana (Louisiana Department of Agriculture, 1998). During and after the harvest each year sugarcane farmers eliminate crop residue by open air burning. This is done for two reasons: (1) to remove fibrous content which would greatly reduce milling efficiency and decrease profits and (2) at 3–10 tons/acre, this post-harvest residue in the field is an impediment to various farming practices. Presently, there is no alternative to burning this residue for the farmer. The smoke from this open air burning contains respirable particles of < 10 μm in size (PM_{10}) (Givens, 1996). PM_{10} is the major cause of respiratory ailments such as asthma and emphysema (Thurston et al., 1994; Schwartz et al., 1993). This open air burning also introduces carbon monoxide and some nitrogen dioxide. A study conducted in California reported an increase in asthma hospitalizations after post-harvest rice residue burning. This study reported that rice burning has a statistically significant effect on asthma morbidity in Butte County, CA (Jacobs et al., 1997).

The current practice of open air burning of post-harvest sugarcane residue is under very close scrutiny by various regulatory agencies because of the recent amendment to the clean air act of 1998. The amendment stipulates that release at the source of particulate matter greater than 2.5 μm is prohibited, whether it is from industry or open air burning. Currently, the open air burning of sugarcane crop residue is exempted from this regulation (Louisiana Department of Environmental Quality, 2000). Due to ever increasing residential development close to sugarcane fields and smoke-related public health risks associated with open air burning of sugarcane crop residue, the indefinite continuance of this exemption is in doubt. The sugarcane growers are currently

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facing increasing pressure to find an alternative method to the current practice of open air burning to solve the crop residue problem. In this study, we investigated a biological method of accelerating decomposition of crop residue and humification of cellulosic material in the soil using Sybron Chemicals consortium of fungus and bacteria. The main objective of this investigation was to find an alternate biological method to the current practice of open air burning of crop residue by sugarcane growers. The specific objectives of the project were the following: (1) To investigate the biological degradation and humification of sugarcane crop residue by the microorganisms provided by Sybron Chemicals, Inc., (2) to optimize the conditions for maximum degradation and humification of sugarcane crop residue, and (3) to evaluate the proposed method and recommend future application of this method in the field.

2. Materials and methods

2.1. Microbial consortium

Sybron Chemicals, Inc. provided the microorganisms for the study. The microbes used in the study were the fungus *C. subvermispora* and bacterium *Cellulomonas* sp., which were chosen for their cellulolytic capabilities and *A. brasilense*, which was chosen because it is a nitrogen-fixing bacterium. It was thought that the presence of bioavailable nitrogen would aid in overall microbial biodegradation rates.

2.2. Procedure

The experiment was conducted in the greenhouse using nine large plastic containers ($58 \times 43 \times 18$ cm). Each container was filled with 4 kg of agricultural soil collected from the Rebecca Farm of Thibodaux, LA, and 1 kg of sugarcane crop residue collected from the same site. The treatments studied were control, microbial consortium sprayed over the residue, and the microbial consortium sprayed over the residue and mixed with soil. Each condition variable was conducted in triplicate. Containers 1–3 were control samples and received 1.5 l of deionized water every month. Containers 4–6 were sprayed with 250 ml *C. subvermispora* (concentration: 8.4×10^5 propagules/ml) and 100 ml each of *A. brasilense* (concentration: 1.3×10^9 CFU/ml) and *Cellulomonas* sp. (concentration: 2.2×10^9 CFU/ml). These containers also received a one-time application of 1.5 l of 0.3% molasses as substrate for the consortium. Containers 7–9 received an identical amount of microbial consortium and molasses as those of containers 4–6, but these pans were mixed thoroughly with soil and repeatedly mixed once a month to provide aeration.

Bacterial and fungal counts in the soil were monitored periodically using pour plates of tryptic soy agar (Brock and Madigan, 1991) and rose bengal agar with streptomycin media (Martin, 1950), respectively. Soil ammonia, nitrate, phosphorous, pH, and total organic matter were analyzed by Hach method (Hach, 1999). Residue breakdown was monitored by visual observation.

3. Results and discussion

The total quantity of aerobic bacteria present in the soils is given in Fig. 1. The bacterial population is significantly higher in the treatment where the microbial consortium was added and the soil and residue were mixed than in the control and in the treatment where the soil was not mixed. The latter two conditions showed relatively low bacterial counts and were virtually unchanged throughout the study period. In the mixed soil and residue samples, bacterial counts peaked and remained constant for most of the study at approximately 2×10^8 CFU/g of soil and began to fall towards the end of the 100 day monitoring period. These results suggest that the molasses and crop residue have been microbially metabolized or that cellulolytic fungal populations are beginning to dominate and suppress bacterial numbers through exclusion. This outcome may be observed in the fungal counts over the 100 day period (Fig. 2). Note that fungal counts in the mixed soil and residue samples increased to 1×10^7 propagules/g of soil and remained constant at the end of the study.

The nutrient content of the soil was periodically monitored. Soil nitrate in the control remained essentially constant throughout the study. In the treatment where the consortium was mixed, the nitrate concentration dropped during the first half of the study and levelled off during the second half of the study (Fig. 3). These data are more clearly understood when viewed alongside the soil ammonia concentrations (Fig. 4) throughout the study. Soil ammonia

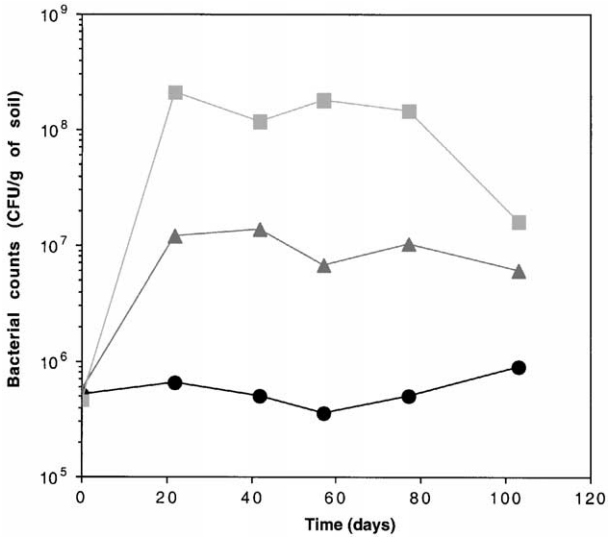

Fig. 1. Bacterial colony forming units (CFU)/g of soil. -●- represents control samples which received monthly watering only; -▲- represents molasses and microbial consortium added but not mixed with soil; -▨- represents molasses and microbial consortium added and mixed with soil.

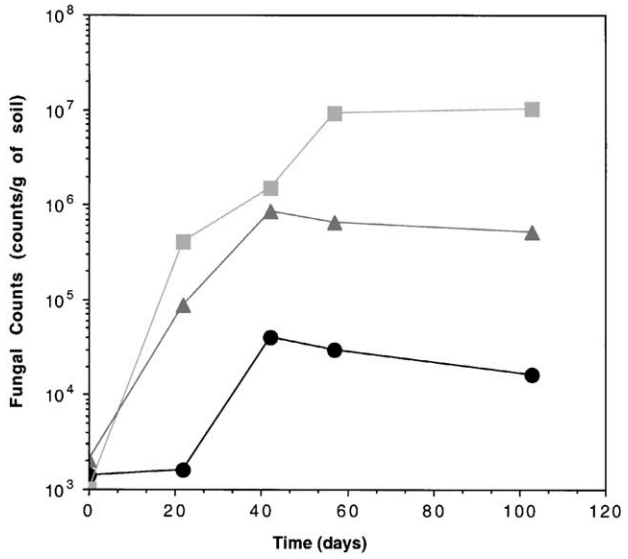

Fig. 2. Fungal propagules/g of soil. -●- represents control samples which received monthly watering only; -▲- represents molasses and microbial consortium added but not mixed with soil; -▨- represents molasses and microbial consortium added and mixed with soil.

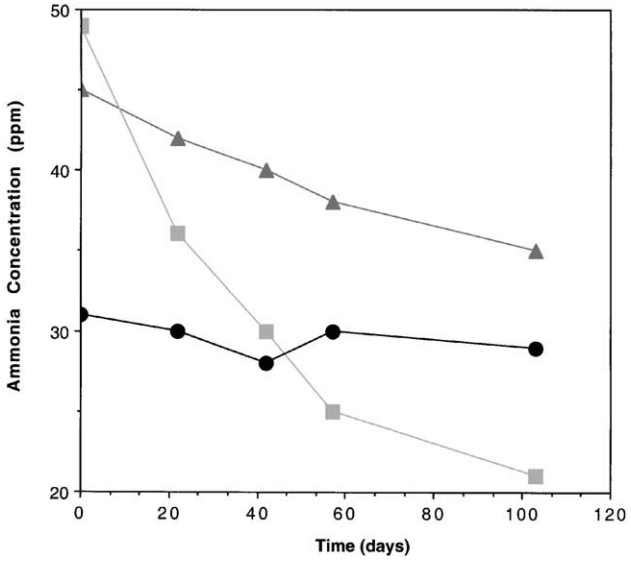

Fig. 4. Soil ammonia concentration as parts per million (ppm). -●- represents control samples which received monthly watering only; -▲- represents molasses and microbial consortium added but not mixed with soil; -▨- represents molasses and microbial consortium added and mixed with soil.

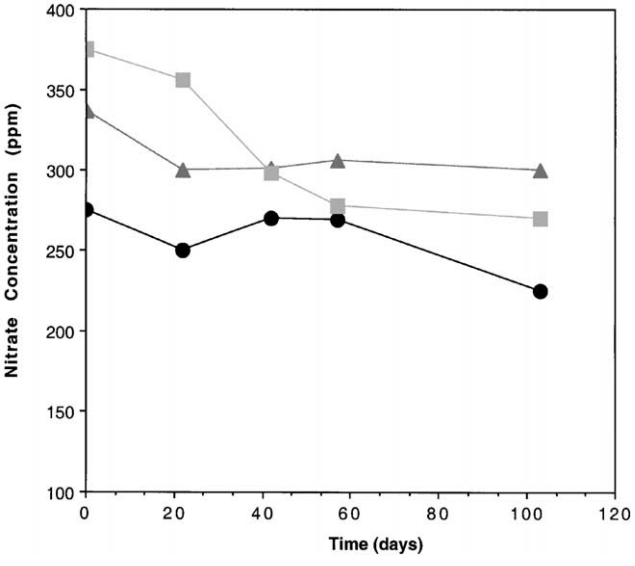

Fig. 3. Soil nitrate concentration as parts per million (ppm). -●- represents control samples which received monthly watering only; -▲- represents molasses and microbial consortium added but not mixed with soil; -▨- represents molasses and microbial consortium added and mixed with soil.

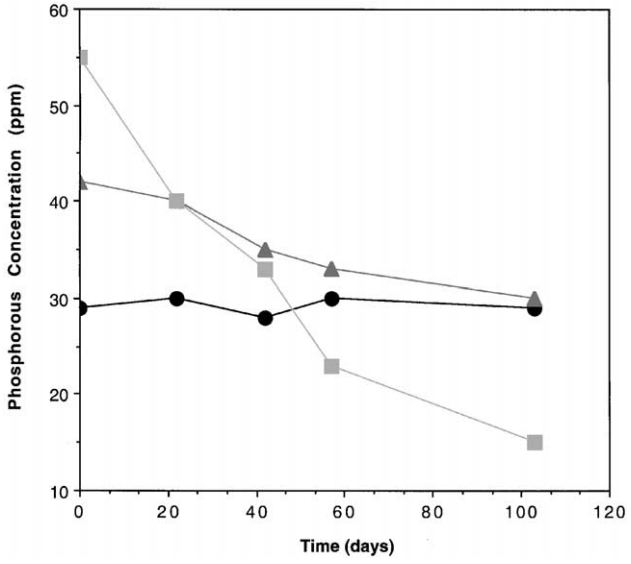

Fig. 5. Soil phosphorus concentration as parts per million (ppm). -●- represents control samples which received monthly watering only; -▲- represents molasses and microbial consortium added but not mixed with soil; -▨- represents molasses and microbial consortium added and mixed with soil.

concentrations in the mixed soil and residue samples dropped sharply and continuously throughout the 100 day time period of the study. These results are consistent with the process of nitrification where microbes aerobically oxidize ammonia to nitrite and eventually to nitrate. The ammonia and nitrite are used as electron donors. During nitrification, ammonia levels would be expected to continuously drop while nitrate levels might be expected to level off as ammonia is converted to nitrate. Nitrification is an aerobic process and occurs in well-oxygenated and drained soils. These samples were kept aerobic by turning over the soil residue mix once a month. Microbial growth rates are kept high by allowing the soil microbes' maximum access to the cellulose substrate that is kept moist by its contact with soil. Microbial contact and substrate moisture are two

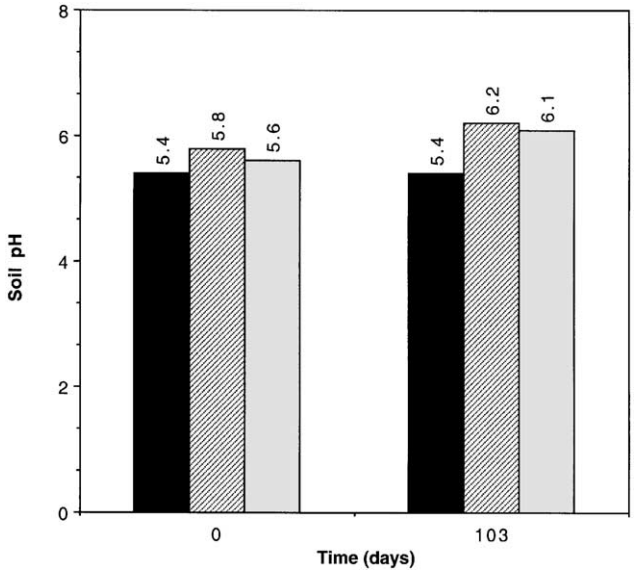

Fig. 6. Soil pH. ■ represents control samples which received monthly watering only; ▨ represents molasses and microbial consortium added but not mixed with soil; □ represents molasses and microbial consortium added and mixed with soil.

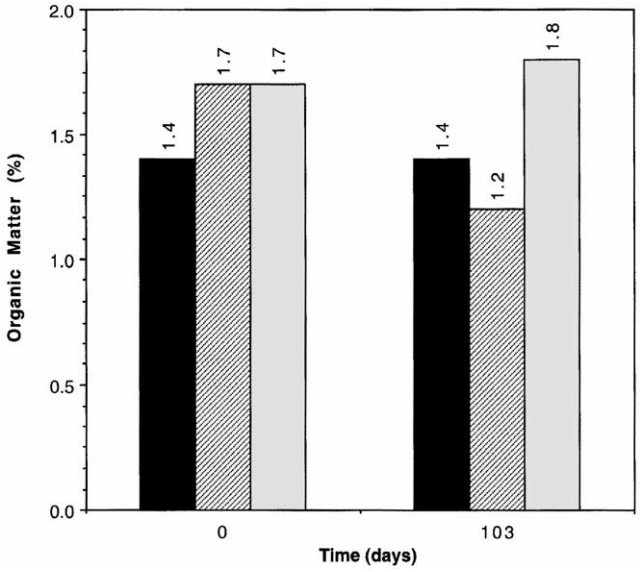

Fig. 7. Soil % organic matter. ■ represents control samples which received monthly watering only; ▨ represents molasses and microbial consortium added but not mixed with soil; □ represents molasses and microbial consortium added and mixed with soil.

factors that help facilitate residue microbial consumption. In the treatment where the soil was not mixed there was only a slight drop in the nitrate concentration. These results are likely to represent the low overall microbial growth that occurred when residue and soil were not mixed and in the control samples. When soil and crop residue are not mixed two factors affect microbial decomposition of cellulose. Those are (1) minimal contact between microbial consortium and the cellulose substrate and (2) the crop residue dries out rapidly and completely leaving little or no available water for microbial metabolism. Soil phosphorous levels were also monitored (Fig. 5).Phosphorus consumption is directly proportional to microbial consortium growth rates as microbes use phosphorus to produce DNA, RNA and ATP. In the treatments with microbial consortium added and where the soil and residue were mixed the soil phosphorus concentration dropped continuously throughout the study period as phosphorus is removed from the soil and stored in living cells. Soil phosphorus dropped little or not at all when soil and residue were not mixed and in control. Again, this is likely directly correlative to lower microbial growth.

During the study period soil pH did not change in control, whereas the pH increased in the non-mixed treatment by a pH unit of 0.4 and in mixed soil and residue treatment by a pH unit of 0.5 (Fig. 6). These increases in soil pH were likely due to increased growth of soil microbes, especially aerobic growth. It is known that during the highly efficient aerobic metabolism of carbohydrates little or no organic acids are made. But in the less efficient anaerobic fermentation of carbohydrates much energy is left unharvested in the form of waste organic acids. It is also thought that any organic acids that were already present in the usually anaerobic soils of south Louisiana would be metabolized as intermediates of the Krebs cycle or converted to those intermediates and fully metabolized. The organic matter content of soil is given in Fig. 7. Soil organic matter for all conditions remained relatively constant during the study. The relatively minor changes observed in percent organic matter of soil are likely explained as being within the experimental error of the procedure. It is thought that repeated microbial degradation of sugarcane crop residue over several years would cause soil organic matter to steadily increase. The end of study visual observation of residue indicates significant physical break up of sugarcane crop residue in the treatment where molasses and microbial consortium were added and soil and residue mixed (Fig. 8). In control and the treatment where molasses and microbial consortium were added but the soil and residue were not mixed, there was no obvious visible breakdown of residue (Fig. 9). The control samples also had no obvious breakdown of residue.

4. Conclusions

Cellulose degradation occurs naturally in complex microbial communities that include many non-cellulolytic organisms (Mullings and Parish, 1984). It is therefore likely that the Sybron bacterial–fungal consortium helps degrade sugarcane crop residue as part of a larger free-living consortium found in sugarcane field soil. This laboratory study provides evidence that the Sybron microbial consortium

Fig. 8. Treatment with molasses and microbial consortium added and mixed with soil.

Fig. 9. Treatment with molasses and microbial consortium added but the soil and residue were not mixed. Control samples were identical in appearance to this treatment.

mixed in soil helped accelerate the decomposition process of sugarcane crop residue. The treatment where the consortium was sprayed over the residue but not mixed in with the soil was not significantly effective. In the control, no significant degradation of sugarcane crop residue was observed. This preliminary study provides evidence that the consortium may work in accelerating the decomposition of sugarcane crop residue, provided the residue is mixed with the soil. It is thought that increased decomposition of sugarcane crop residue occurred for three reasons: (1) the presence of molasses provided a readily available source of carbon, proteins, nitrogen and vitamins to allow an initial rapid increase in total microbial numbers, (2) increased contact between soil and residue allows increased mass transfer from crop residue to microbial cells, and (3) increased contact between soil and residue allows the residue to remain wet throughout the study thus allowing microbial metabolism to occur. Further study is needed to extend this work for possible future application of this technology. Future study should be directed at the use of consortium in various forms such as fungal spores, hyphae, active cultures, lyophilized bacteria and liquid cultures.

Future field studies should be conducted using these various microbial forms with the goal of optimizing shelf life and ease of application for farmers. Also, a long-term study should be done to examine what effect herbicide and fertilizer applications may have on performance of the Sybron microbial consortia.

Once the sugarcane stalk is harvested, the next year's crop emerges from the sugarcane stalk stubble that is left in the ground. What effect, if any, the microbial consortium may have on emerging sugarcane stubble should also be studied. It is doubtful that microbes that have evolved to be plant residue decomposers would have any negative effect on living plants but this should be verified scientifically. This would ensure farmers that application of these cellulolytic microbes causes no harm to subsequent years' crop yield. Preliminary results indicate quite the opposite. Agricultural soils subjected to this treatment for several years would likely acquire a significant layer of organic matter that would lessen the need for commercial fertilizer applications. This would represent an increase in soil fertility and lead to maintained or increased crop yields while lessening external fertilizer applications. Increased organic matter would also increase soil porosity, aeration and fertilizer retention (Widrig et al., 1997). This study suggests that mixing this microbial consortium and molasses into soils would lessen the acidity of the soil. It is likely that this would also aid in increasing crop yields. A 5-year study (Golden, 1972) where acidic soil was amended closer to neutral pH increased sugarcane tonnage yielded per acre by 7% and pounds of sugar per acre by 6%. Golden also stated that when soil pH was below 6.0, as was the case in our study, amendment of soil pH closer to neutral would have a significant positive impact on sugarcane yield.

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