Extraction of Energy Precursors in the Form of Volatile Fatty Acids (VFAs) from the Xerophyte Prosopis *(Prosopis juliflora)*

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1 Introduction

Prosopis *(Prosopis juliflora)* is a xerophyte native to harsh desert environments [1]. In that settling, it serves very useful purpose due to its extreme hardiness and survivability. But when it was taken to countries outside its region of origin, it has become weedy. The very same attributes due to which it can thrive in extremes of climate and resource shortage enable it to outcompete most other vegetation in regions of better climate and land-water-nutrient availability. This poses great risk to biodiversity [3].

India—especially its tropical and subtropical parts—is facing a great onslaught of Prosopis [1]. The situation in some regions like Tamil Nadu has become so bad that High Courts have intervened to order the government to clear the landmasses from Prosopis [1, 2].

The stem and the woody branches of Prosopis are usable as fuelwood. Indeed Prosopis fuelwood is in demand at restaurants because it is believed to impart special taste to food roasted over its fire, but the Prosopis leaves have no utility. When rain falls on them, or in moist soil, they keep leaching out allelopathic chemicals which toxify the soil and discourage other species of vegetation [4].

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Unlike other weeds [5–8], they biodegrade very slowly due to which they exert their negative impacts over a long time span. This raises the urgent need to find ways to utilize Prosopis leaves so that they can be utilized when they fall from live trees or are obtained when Prosopis trees are chopped for wood.

In this chapter, attempts to generate energy precursors from Prosopis leaves in the form of volatile fatty acids (VFAs) are described. The VFAs are directly utilizable to generate energy in the form of biogas as has been illustrated in Chapter 28 of this volume [9].

2 Materials and Methods

All chemicals were analytical regent grade unless otherwise specified. Alkali-resistant glassware and deionized, double-distilled water were used for all analytical work.

Prosopis leaves were collected from healthy, adult Prosopis trees standing near the Pondicherry University campus. After the leaves were rinsed with water and wiped—to free them from attached dust and invertebrates—their dry weight was determined. For this, fresh weight of three separate randomly picked samples was taken, and the samples were then oven dried at 105 °C to a constant weight. The reactors were inoculated with fresh cow dung because fresh cow dung contains cellulolytic, acidogenic, and acetogenic bacteria that are needed to biodegrade Prosopis. The cow dung for this purpose was obtained from a nearby dairy. Its dry weight was also determined at 105 °C. For all the computations of the VFA yield, dry weight of cow dung and Prosopis leaves has been used as the basis.

The reactors used for extracting VFAs were comprised of 15 L plastic containers. They had a tap at the bottom with which the contents at the end of each experiment can be drained off.

Two sets of reactors, with or without Prosopis, were operated. The first set, with reactors designated as R1A to R6A, had the following content:

R1A Prosopis 1.5 kg + 12 L water inoculated with 1% cow dung
R2A As above but without Prosopis
R3A Prosopis 1.5 kg + 12 L water inoculated with 2.5% cow dung
R4A As above but without Prosopis
R5A Prosopis 1.5 kg + 12 L water inoculated with 5% cow dung
R6A As above but without Prosopis

The second set, designed as R1B–R6B, was identical and served as the duplicate. The Prosopis content of 1.5 kg fresh leaves was equivalent to 417 g dry weight in all Prosopis-fed reactors.

As the reactors were to be kept in the acid phase [10, 11] which is based on aerobic and facultative bacteria, it was essential that anaerobic conditions were prevented from being developed in the reactors. To enable this, the reactor surface was kept in contact with air by not putting a lid over it but only covering it with a

nylon mesh to keep off insects. Also the reactor contents were mixed once every 8 h manually using a fiber glass shovel.

After 24 h had elapsed from the time of starting the reactors, the first sample was drawn. For it, the contents were first stirred to homogenize them. After coarse solids settled in about 10 min, four 25 mL aliquots were pipetted out from four randomly picked locations in the reactors, differing from each other in position at the surface and the depth. The samples were pooled. The reactors were compensated for the 100 mL of reactor content taken out for sampling by adding 100 mL of distilled water to the contents. The sample was then placed in a 500 mL round-bottomed flask kept on a heating mantle, and 100 mL of water with 5 mL concentrated H_2SO_4 was provided to it. A few pieces of broken glass were added to prevent thermal stratification by facilitating agitation. A condenser was then fixed and distillation was carried out at a rate of about 5 ml of distillate condensing per minute. The first 3 min (about 15 mL) of distillate was discarded and the distillation resumed to completion.

After the receiving flask was detached from the condenser, the VFA concentration of its contents was determined by titration with a 0.02 N sodium hydroxide solution using phenolphthalein as indicator [12]. As the procedure is highly sensitive to the shape of the glassware, precise quantum of heat provided, and similar other factors, a calibration curve correlating VFA concentration actually taken with the concentration arrived after distillation was drawn up. This was done on the basis of a large number of runs in which a range of known acetic acid concentrations was 'recovered' by distillation.

The experiments of Series A were completed first. The reactors were then cleaned and the entire experiment was repeated using a fresh harvest of Prosopis and freshly acquired cow dung for the duplicate Series B. This was done to enable us to test the reproducibility *vis-a-vis* VFA extraction carried out with different harvests of Prosopis, different sources of cow dung inoculum, and at different times.

3 Results and Discussion

The findings are summarized in Tables 1, 2, 3, and 4. The contribution of only cow dung in VFA generation is reflected in Table 1. As expected, the highest blank occurs in reactors with 5% cow dung but the average VFA generated by this blank —542.4 mg/L—is about a fourth of the yield in Prosopis—charged reactors of which average is in the range 2180–2415.7 mg/L (equivalent to 49.9–71.7 g/kg as in Tables 2, 3, and 4). In reactors with 1% cow dung as inoculum, the blank (average 81.4 mg/L) is insignificant in comparison with the VFA generated in presence of Prosopis (2296–2492 mg/L).

Tables 2, 3, and 4 reveal that even as VFA generated on different days in the duplicate reactors has wide variation, the average yields are remarkably similar. It is also seen that by the third day in reactors inoculated with 1% cow dung (Table 2) and 2.5% cow dung (Table 3), the VFA yield reaches near the overall average

Table 1 VFA generated by different concentrations of cow dung inoculum

Number of days from the start of the reactor	VFA generated, mg/L, by cow dung		
	1%	2.5%	5.0%
2	36	210	336
3	66	234	546
4	66	288	618
5	99	336	627
6	126	360	657
7	144	417	708
8	165	490	194
9	48	498	624
10	54	384	510
11	78	432	588
12	30	339	564
13	180	351	648
14	72	297	654
15	60	276	621
16	39	258	522
17	48	249	480
18	72	246	234
Avg ± SD	81.4 ± 45.8	333.2 ± 88.1	542.4 ± 139.1

Table 2 Volatile fatty acids (VFAs) generated by duplicate reactors in treatment R1

Number of days from the start of the reactor	VFA yield, g/kg	
	R-1A	R-1B
2	38.7	31.4
3	54.6	50.2
4	69.9	59.9
5	70.5	63.1
6	72.4	65.4
7	82.1	76.4
8	107.2	71.5
9	90.8	80.0
10	84.4	80.6
11	84.8	71.0
12	86.3	73.5
13	79.3	69.1
14	57.8	79.1
15	69.2	48.9
16	78.4	45.5
17	42.1	83.3

(continued)

Table 2 (continued)

Number of days from the start of the reactor	VFA yield, g/kg	
	R-1A	R-1B
18	51.0	34.5
Avg ± SD	71.7 ± 18.2	63.7 ± 16.2
Overall avg ± SD	67.7 ± 14.8	

Table 3 Volatile fatty acids (VFAs) generated by duplicate reactors in treatment R2

Number of days from the start of the reactor	VFA yield, g/kg	
	R-2A	R-2B
2	27.8	26.6
3	45.9	41.0
4	55.0	54.0
5	52.7	58.4
6	54.0	60.6
7	64.4	64.5
8	57.9	62.8
9	58.7	67.5
10	60.2	62.0
11	43.3	60.1
12	64.5	61.2
13	70.7	63.7
14	57.8	59.9
15	55.8	57.7
16	60.4	60.5
17	54.3	56.7
18	53.0	50.2
Avg ± SD	55.1 ± 9.6	56.9 ± 9.9
Overall avg ± SD	56 ± 9.3	

value. The same happens by fourth day in reactors with 5% cow dung (Table 4). In subsequent days, the VFA levels hover around the overall average. The constancy in VFA levels over the 18-day span of experiments also indicates that either there is no microbical destruction of the VFAs that are generated or the destruction, if it occurs, is compensated by the formation of fresh VFAs.

The average VFA generation with three levels of cow dung as a function of time is depicted in Fig. 1. Whereas reactors with 1% inoculum have given clearly better yield on all days, except the 18th, the performance of reactors with 2.5 and 5% inoculum is not so sharply distinct from each other. Yet it is clear that VFA generation follows the order 1% > 2.5 > 5% *vis-a-vis* inoculum concentration. The difference pertaining to 1% and the other two inoculum concentrations is

Table 4 Volatile fatty acids (VFAs) generated by duplicate reactors in treatment R3

Number of days from the start of the reactor	VFA yield, g/kg	
	R-3A	R-3B
2	28.0	23.8
3	32.0	31.8
4	40.9	38.9
5	48.4	39.8
6	48.4	41.1
7	56.8	51.1
8	68.2	66.9
9	55.3	57.5
10	57.8	61.3
11	61.0	62.6
12	71.8	42.3
13	53.7	57.8
14	49.0	52.8
15	41.6	51.7
16	57.5	62.8
17	43.9	61.5
18	53.2	44.6
Avg ± SD	51 ± 11.5	49.9 ± 12.3
Overall avg ± SD	50.5 ± 10.8	

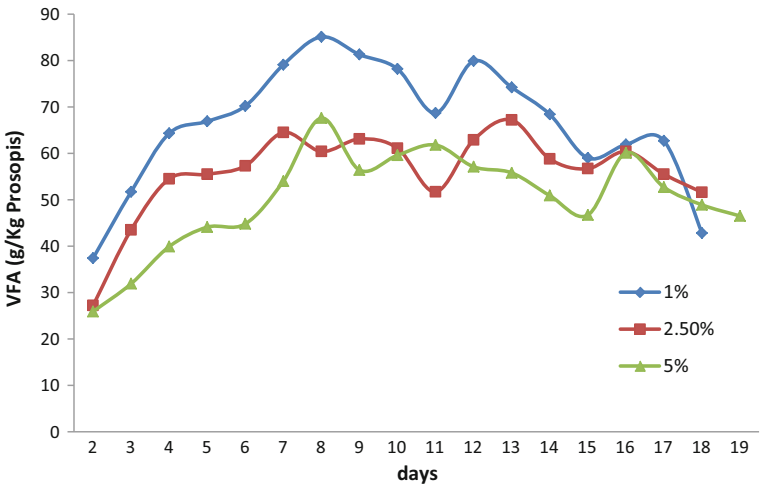

Fig. 1 VFA generated by Prosopis as a function of time in reactors inoculated with 1, 2.5, and 5% cow dung

statistically significant at confidence level >95% while the difference between the influence of 2.5 and 5% inoculum is not statistically significant. This seems to suggest that cow dung may be hindering, rather than facilitating, VFA generation.

The maximum VFA generation recorded was 107.2 g/kg (Table 2), representing about 11% conversion of Prosopis leaves into VFAs. This finding suggests that pretreatment of Prosopis by NaOH, HCl, or other chemicals known to hydrolyze some of the polymeric sugars to simpler (and more easily biodegradable) molecules [1] may lead to greater than 11% of Prosopis yielding VFAs.

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