# Mushroom cultivation on spent biomass from biogas plants

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**Mushroom cultivation on spent biomass feedstocks from biogas plants is an attractive option to utilize the residual lignin and cellulosics to create cash flow from the use of biogas plants and make their use and operation lucrative in villages. The cultivation of oyster mushroom, *Pleurotus flabellatus*, on spent biomass feedstock from biogas plants has been shown to be feasible in this study. The substrate preparation techniques as well as the nature of biomass feedstock used for biogasification are shown to have a marked influence on mushroom growth and yields. The greater availability of protein in spent biomass feedstock and straw mixture appears to be responsible for over 150% increase in mushroom yields and therefore is an attractive area for further investigations.**

THERE are about 1.5 million small and medium-sized biogas plants in India[1] and these are reported to be economically viable when they replace firewood at commercial prices[2]. However, most rural households gather firewood at zero private cost[3] and hence the benefits of biogas plants appear notional to them unless techniques to derive perceptible monetary returns directly from the use of biogas plants are developed and promoted[4]. Owing to the shortage of dung in rural areas several small-scale biogas plants operating on non-dung biomass feeds are being developed for rural use[5] and these are also likely to suffer low acceptance unless cash flow accrues from their use. Spent biomass feedstocks from biogas plants retain 40–60% of the cellulose and lignin[5] thus providing adequate potential for the cultivation of edible mushrooms and to generate cash outflow by the use of biogas plants in rural areas. In addition, the skill and technology to cultivate and market mushroom is now available in rural India.

Paddy straw is considered one of the best substrates for oyster mushroom (*Pleurotus* sp.) production[6]; replacing paddy straw without sacrificing yields is therefore difficult. The use of alternate substrates often results in reduced yields, high bacterial activity[7] and also contamination by other fungi. Cultivation of oyster mushroom on biogas plant's spent residue has not been reported before. An attempt was made to determine the feasibility of mushroom cultivation on spent biomass from biogas plants and to identify substrate preparation techniques needed to cultivate oyster mushroom on this substrate. Oyster mushroom was chosen for this study as it requires less stringent conditions and tolerates the warm climate[8] of South India. Leaf biomass-based biogas plants use dilute biogas slurry only during the start-up and therefore run very low risks of pathogen transfer during continous operation and consequently would pose low health risks.

Spent material from biomass-based biogas plants being an hitherto untested mushroom substrate, an attempt was made here to first determine the feasibility of using such a substrate and secondly to develop necessary modifications to the existing cultivation techniques. Various proportions of spent biomass and paddy straw were used as raw material to cultivate oyster mushroom by the conventional polythene bag method[9, 10]. Spent feed from an experimental 12 $m^3$ 'plug flow' biogas

**Table 1.** Pretreatments used and their effects on mushroom growth and yields

| Treatment/performance | Trial 1 | Trial 2 | Trial 3 | Trial 4 |
|---|---|---|---|---|
| Starting date | Oct 1 | Oct 8 | Nov 10 | Nov 18 |
| Predominant biomass species in spent biomass | *Euphorbia* sp. | *Euphorbia* sp. | *Euphorbia* sp. | *Syndrella* sp. |
| Wet weight/bag, (kg) | 2.4 | 2.4 | 2 8 | 2.8 |
| Dry weight (kg) | 0.4 | 0.4 | 0 440 | 0.440 |
| Dry weight (kg, straw[2] + kg, biomass[2]) | na | na | 0.220 + 0.215 | 0.220 + 0 212 |
| Biomass : straw ratios (per cent straw) | 0,50,100 | 0,50,100 | 0,50,100 | 50 & 100 |
| Pretreatment[3] used | A | OD | AD + SS | LOD + HW |
| Mushroom yields[4] | 0.412 | 0 400 | 0.423 | 0.452 |
| a) 100% straw | | | (0 961) | (1.027) |
| b) 50% biomass layered | – | – | 0.342 | 1.250 |
| | | | (0.786) | (2.894) |
| c) 50% biomass mixed | – | – | | 1.075 |
| | | | | (2 488) |

1–Only paddy straw.
2–50% each of paddy straw and biomass, dry weight basis.
3–Pretreatments: A – autoclaving 15 bar 60 min, OD – oven drying, 16 h @ 80°C, SS – steam sterilized at 1 bar 95°C for 2 h, LOD – long duration oven drying 80°C 150h, HW – hot water rehydration, AD – air dried for 15 d
4–Yields fresh mushroom weight/bag. Values in parenthesis (kg fresh mushroom/kg dry substrate).

plant (mixed leaf biomass feedstock) was used as raw material for mushroom cultivation. Spent biomass is expected to carry a large load of undesirable microorganisms (Ramasamy K, 1992, unpublished report) and therefore four simple pretreatment methods to sterilize this substrate were tried and one method gave high mushroom yields.

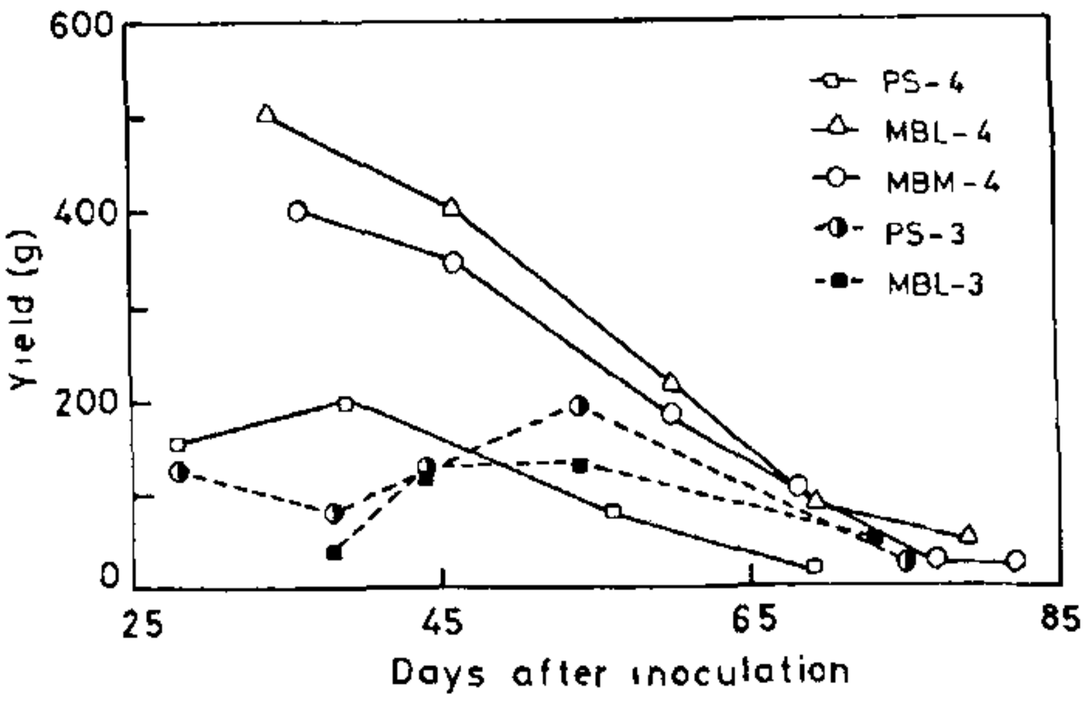


**Figure 1.** Fresh weight of mushroom harvested from different substrates and their combinations; paddy straw control (PS), spent biomass + paddy straw (1:1)) placed in layers (MBL), and well mixed (MBM), Numeral indicates trial number.

Mushroom cultivation was attempted in a total of four trials conducted between October 1992 and March 1993 under laboratory conditions (21–26°C) in a simple humid chamber covered with moistened jute cloth. Three combinations of spent biomass feed and straw mix were tried (0, 50 and 100%). The pretreatments tried were (i) autoclaving, (ii) oven drying (80°C for 16 h), (iii) steam sterilizing (95°C for 2 h) air dried material, (iv) rehydrating oven dried material (80°C for 150 h), for trials 1–4, respectively.

Spent biomass feedstock was recovered from an experimental plug flow like digester (digester capacity 12 $m^3$) operating on leaf biomass feedstock with a residence time of about 40–50 d. During trials 1–3 the feed material comprised of land weeds such as *Euphorbia notoptera* (50%), *Synedrella nodiflora* (30%) and the remaining portion of mixed species while the predominant feedstock during trial 4 was *Synedrella nodiflora* (> 80%). Spent feed was normally allowed to drain free

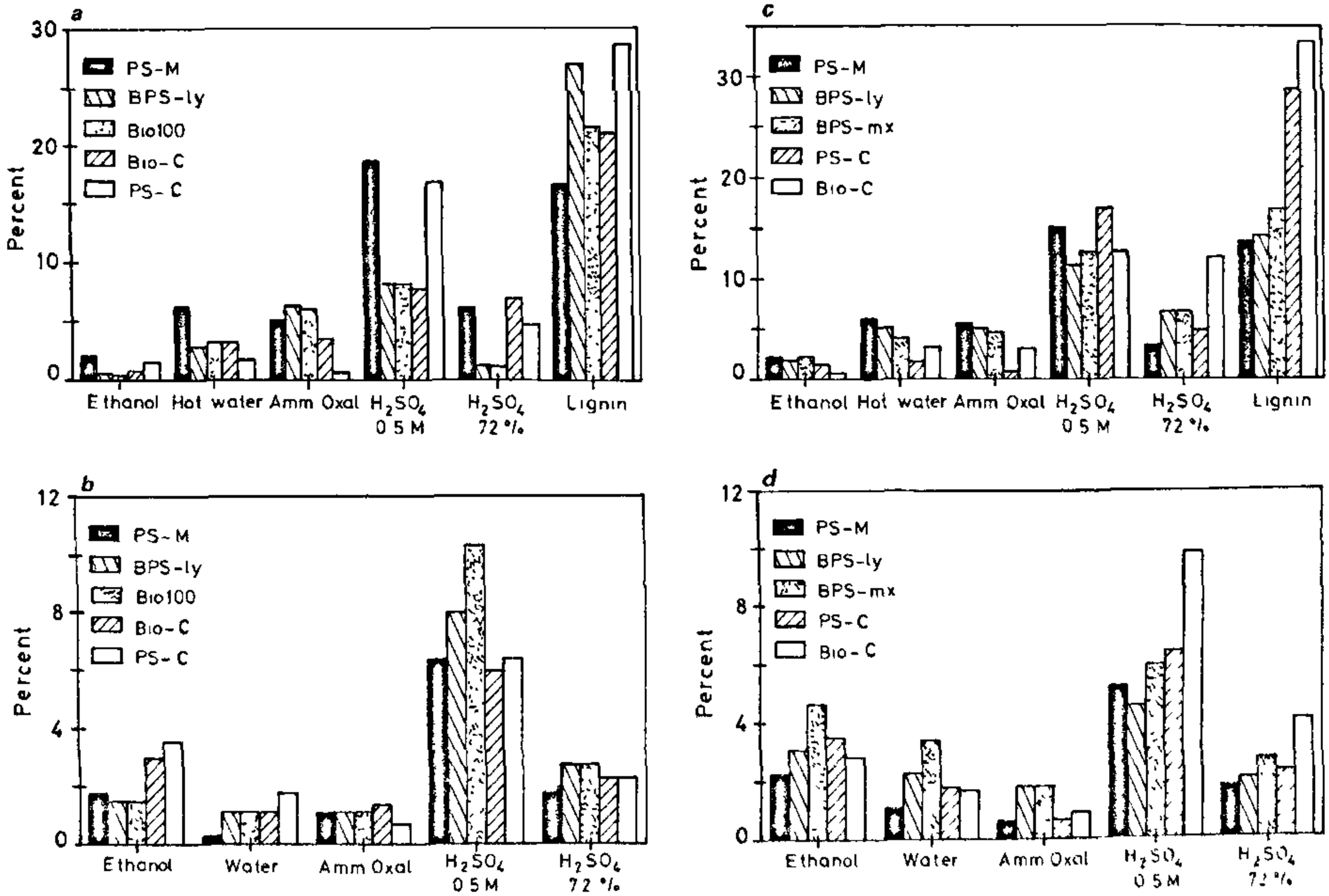


**Figure 2.** Changes in composition of substrate after mushroom growth (2A and B represent results of trial 3, 2C, D trial 4) Legends – PS-C = paddy straw control (unmoculated), BPS Ly – biomass and paddy straw (1 : 1) in layers, BPS-mx = biomass and paddy straw (1 : 1) thoughly mixed, Bio100 – 100% spent biomass, Bio-C-spent biomass control (unmoculated). PS-M – spent residue after mushroom growth on paddy straw

of digester liquid and used as mentioned above. The mushroom yields are reported as fresh weight of mushroom harvested in 80 d per unit fresh and dry weights of substrates. As straw was allowed to decompose longer without mushroom yields, meaningful comparison of bioefficiency could not be undertaken.

In all the four trials, substrate for the 100% paddy straw control was prepared by the hot water dip method[9,10]. The changes in composition of substrate following mushroom cultivation were determined by sequential solubilization with different extractants[11]. The extractants used were as follows, petroleum ether for fats and lipids; hot ethanol — free sugars and proteins; hot water — pectins and simple proteins; hot ammonium oxalate — bound pectins and proteins; 0.5 M $H_2SO_4$ — hemicellulose and proteins; 72% $H_2SO_4$ — cellulose and the remaining residue lignin was corrected for ash. The sugar and protein contents in different fractions were determined by phenol sulphuric acid and Lowry's methods, respectively, with appropriate standards. Mushroom spawn (*P. flabellatus*) was obtained from Prof. K. Shivappa Shetty, of University of Agricultural Sciences, GKVK, Bangalore.

The pretreatments tried and the results obtained are summarized in Table 1 and Figures 1 and 2. During the first two trials, mycelial growth progressed well in bags containing 100% spent biomass. However, these beds collapsed (compacted) and were infested by insects and other fungi after about 14 d of spawn run giving little chance for further mycelial growth and fruit body development. It was found that spent biomass feedstock, when autoclaved, became very soft, lost its stiffness and the cultivation bed collapsed resulting in a compacted material not suitable for mushroom cultivation. Paddy straw control gave a little above 400 g mushroom/bag during these two trials. Short duration drying at 80°C or autoclaving of feedstock[12] was therefore an inadequate pretreatment technique for this substrate and resulted in poor aeration and contamination.

The problem of biomass bed collapsing during spawn run needed to be overcome by mixing spent biomass with another substrate. Spent biomass and paddy straw added in alternate layers (4 cm) arrested this collapse and retained adequate aeration and structure. The spent biomass used in this trial (trial 3) contained mainly *Euphorbia* sp and *Synedrella* sp. During this trial biomass and straw mixture (1 : 1) and paddy straw control gave similar mushroom yields (353 and 423 g/bag, Figure 1). The succesful fruiting in trial 3 indicated that spent biomass feed from biogas plant which becomes very soft during sterilization had to be supported by mixing it with a tougher biomass substrate such as paddy straw. Pretreatment by steam sterilization at atmospheric pressure (95°C for 2 h) is a better alternative.

The nature of substrate utilization during trial 3 is presented in Figures 2 *a–c* and the results are summarized as follows.

(i) Free sugars and pectins increased in substrate after mushroom cultivation (Figure 2 *a*, *b*) while hemicellulose and cellulose were similar and lignin content reduced significantly. This pattern suggests an increasing order of preference for these components by this fungus as well as the existence of normal cultivation conditions and absence of stresses.

(ii) Biomass : straw mixtures (1 : 1 in layers or 100% spent biomass) yielded a spent residue of a composition similar to the above (Figure 2 *a*, *b*) except for protein and lignin constituents. Spent biomass had a higher content of protein (and kjeldahl N) while spent biomass and straw mix (1 : 1) gave a higher lignin content indicating incomplete utilization under this treatment. The similar composition of the two substrates after cessation of mushroom growth suggests that growth and substrate utilization reached similar levels in both treatments indicating that the lack of fruiting bodies was not due to problems related to saccharide or lignin utilization.

(iii) Ample mycelial growth was observed with spent biomass alone as substrate but sporophore development did not occur. Spent biomass was more compact than straw : biomass mixture to begin with. Exposed biomass bed during sporophore formation continued to undergo compaction. The lack of fruit body development therefore appears to be related to its compact physical form resulting in poor aeration.

(iv) Cellulose utilization was enhanced and lignin utilization reduced in spent biomass mixtures compared to the paddy straw control (Figure 2 *a*).

The problem of biomass feed undergoing compaction during fruit body development stage could not be completely avoided in trial 3. It was envisaged that longer drying would retard rehydration of the substrate and therefore its rapid softening and concomitant amenability to contamination. Low temperature oven drying (80°C for 150 h) was used as a pretreatment to emulate long-term sun drying in the field. This pretreatment greatly enhanced suitability of the material for mushroom cultivation (trial 4, Figures 1 and 3, Table 1).

Distributing spent biomass and paddy straw either in separate layers (4 cm thick) or by complete mixing gave total yields in excess of 1 kg mushroom/bag as compared to about 0.44 kg/bag for paddy straw control (Figure 1). In spite of a delayed fruiting, mixed biomass substrates gave 5 harvests in 80d against a normal 3 harvests in 45–50 d with paddy straw[6]. The arresting of the biomass bed from collapsing, the biomass type and/or the high protein content of the spent biomass feed may be the cause of abundant fungus growth and yields. Protein-rich materials such as horse gram powder, oat and cotton seed meal, etc. have been earlier frequently used to boost mycelial growth and mushroom yield[6] but causes bacterial rot. The intensity of fruit body production

**Figure 3.** Intense fruit body production on spent biomass + straw placed in 4 cm layers photographed before the first harvest (> 500 g) in trial 4.

on mixed substrate (Figure 3) and the yields obtained here (2.5–2.8 kg mushroom/kg dry substrate) are high and well above that normally reported (1 kg fresh mushroom/kg dry straw)[8]. It is therefore necessary to understand the underlying causes.

The changes in composition of the substrates following mushroom growth are presented in Figures 2 *c, d* (trial 4) and are summarized below.

(i) The growth of *P. flabellatus* on paddy straw brought about an increase in simple saccharides, small changes in hemicellulose and cellulose fractions and a > 50% reduction in lignin content, a pattern similar to that observed in trial 3. A noticeable reduction was observed in all the protein fractions unlike that observed with biomass straw mixtures.

(ii) The method of mixing spent biomass and straw (in layers or completely mixed) did not affect the saccharide utilization pattern.

(iii) A marked increase in simple protein fractions (water and oxalate soluble) and a reduction in complex proteins were observed suggesting a breakdown of complex proteins during growth and sporophore formation in biomass : straw mixtures.

(iv) An increased protein utilization (and kjeldahl N, Table 2), the absence of bed collapsing and the nature

**Table 2.** Changes in kjeldahl nitrogen in the substrates tried (% dry weight)

| Sample | Spent biomass feed | Paddy straw | Biomass Straw mix (1 : 1) |
|---|---|---|---|
| Trial 3: | 0.93 | 0.35 | 0.64[1] |
| Precultivation | 1.33 | 0.35 | 1.33 |
| Post cultivation | | | |
| Trial 4: | | | |
| Precultivation | 1.26 | 0.35 | 0 80[1] |
| Post cultivation | NT | 0.35 | 0.63(L) |
| | | | 0 63(M) |

1–Computed value. NT – not
Letters in parenthesis L – bi[illegible] ers and M completely mixed

of the biomass feedstock (mostly *Synedrella* sp.) are the possible causes for intense fruit body formation and high yields.

This study indicated that cultivation of oyster mushroom (*Pleurotus flabellatus*) on spent feedstocks of biomass- based biogas plants is feasible with simple modifications to the existing technology. There is potential for further improvements in mushroom yields as well as cultivation techniques. The higher yields of mushroom grown on spent biomass substrate could provide greater monetary returns and make the use of biomass-based biogas plants attractive. Such an incentive can greatly assist the application and spread of biogas technology in rural India. The reasons for these high yields and influence of types of spent biomass feedstock, however, needs to be determined. Simpler pretreatment procedures such as solar sterilization, etc. of spent biomass have to be developed and standardized for different kinds of spent biomass feedstocks.


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# Time and duration of Deccan volcanism in Razole area, Krishna–Godavari Basin, India

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KDM Institute of Petroproteins; hot and & Natural Gas Commission, Dehra Dun 248 [illegible]; 0.5

**Nannoplankton data throws light on the time and duration of the Deccan trap occurring in the subsurface section of Krishna–Godavari Basin, India. The results are more refined than the planktonic foraminifera data published in the last three years. Calcareous nannoplankton discovered from the infratrappean sediments are assigned to *Micula murus* zone of the Late Maastrichtian age, which fixes the lower age limit of the trap. The supratrappean sediments mark the upper age limit of the trap and are assigned to upper part of NP2 : *Cruciplacolithus tenuis* zone of upper part of Early Danian age. The duration of Deccan volcanism in this region is estimated to be about 3.8 m.y. and not 6.0 m.y. as suggested by earlier workers in the Krishna–Godavari Basin.**

THIS note deals with the study of calcareous nannofossils in the infratrappean and supratrappean subsurface sediments of the Razole Area, Krishna–Godavari Basin, India. The upper and lower limit of the traps and duration of Deccan Volcanism in this region are discussed.

The Sediments between depth interval 3660–3665 m (infratrappean) occurring below the 300 m thick trap and those immediately overlying the trap between depth interval 3360–3365 m (supratrappean) of Razole-A well are examined for calcareous nannofossils (Figure 1). These sediments yielded rare, but reasonably well-preserved calcareous nannofossils whose distribution is shown in Table 1. The nannofossils are documented under Leitz Orthoplan polarizing microscope using oil immersion objective (Figures 2–26).

The subsurface sediments between the depth interval 3660–65 m below the trap yielded the following nannoplankton species : *Micula murus*, *M. decussata*, *Cyclagelosphaera reinhardtii*, *Prediscosphaera spinosa*, *P. cretacea*, *Stradneria crenulata* and *Arkhangelskiella* sp.

Out of the above nannoplankton species, *Micula murus* first appears at the base of nannoplankton zone NC23[1] and CC26[2] and vanishes at the top of it. The base of zone CC26 practically coincides with the base of *Abathomphalus mayaroensis* zone of planktonic foraminifera. Therefore the first occurrence of *Micula murus* establishes Late Maastrichtian age of the infratrappean.

The sediments between the depth interval 3360–65 m just above the trap yielded the following nannofossils: *Cruciplacolithus primus* (5–8 μ), *Prinsius dimorphosus*, *Thoracosphaera operculata*, *Coccolithus pelagicus*, *Braarudosphaera bigelowii*. Out of these species, *Cruciplacolithus primus* and *Prinsius dimorphosus* are age-diagnostic. Relatively large *Cruciplacolithus primus* (5–8 μ) ranges from NP1 to NP2 zone and *Prinsius dimorphosus* ranges from upper part of NP2 to lower part of NP4. The concurrent ranges of these two species of nannoplankton date these sediments as equivalent to upper part of NP2 : *Cruciplacolithus tenuis* zone of upper part of Early Danian age (written communication Kathorina Van Salis, 1993).

The extensive foraminiferal study of infratrappean, intertrappean and supratrappean beds are made in Palakollu-A, Elamanchili-A and Modi-A wells of Krishna–Godavari basin[3]. It shows the oldest flow was post *A. mayaroensis* zone (66.5 m.y. to 67.5 m.y.). The base of the *A. mayaroensis* zone nearly coincides with the base of *Micula murus* zone. The youngest flow was reported pre P2 zone (60.2 to 61.4 m.y.) of standard

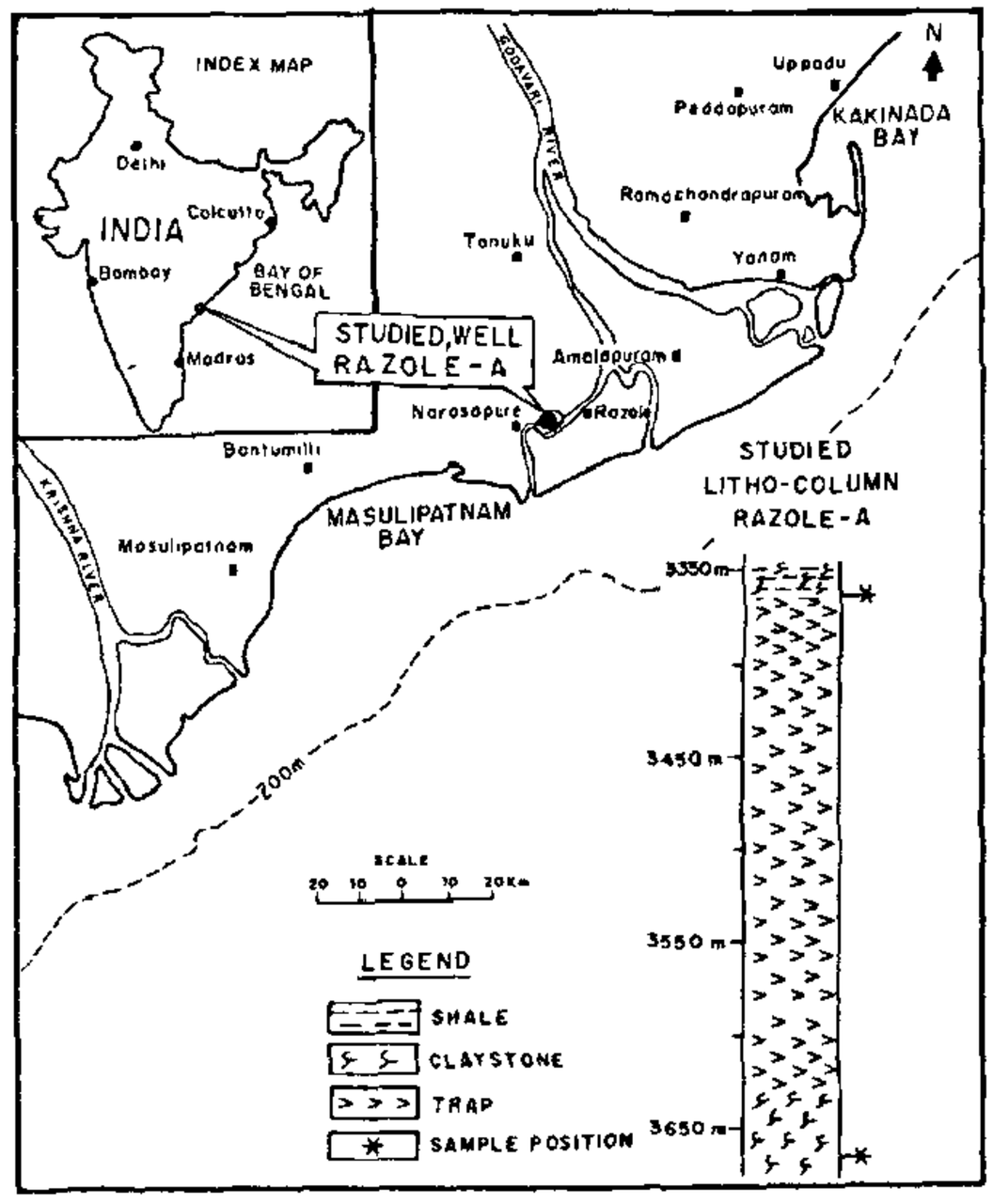


Figure 1. Map showing location of well Razole-A in the Krishna–Godavari Basin. The lithocolumn of Razole-A well between depth interval of 3350 and 3665 m is shown with the sample position marked by asterisk. *Infratrappean sediments* are between 3660 and 3665 m and supratrappean sediments between 3360 and 3365 m depth interval.