Studies on bio-pretreatment of pine needles for sustainable energy thereby preventing wild forest fires

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Deforestation, forest fire hazard and exploitation of forest have been warning threats to our environment and ecosystem globally. In the present study an attempt has been made to enhance the biodegradability and biomethanation potential of treated pine needles, the leaves of a coniferous tree (*Pinus roxburghii*) by using *Trichoderma* spp. and *Pseudomonas* spp. thereby, utilizing the pine needles for green energy. Studies were carried out in four litre capacity polymer reagent bottles as anaerobic bioreactor at mesophilic conditions (35 ± 1°C) for 80 days. The experiments were replicated thrice and the results compared with untreated ground pine needles substrate (control). The results showed a specific biomethane production of 0.269 l/g VS destroyed from bio-pretreated substrates whereas it was 0.133 l/g VS destroyed for untreated substrate with a biodegradability of 10.41% and 4.78% respectively. Results indicate that bio-pretreated substrate produced a cumulative biomethane yield of 21.3 l/kg pine needles which was 285% higher as compared to the untreated pine needles substrate (5.53 l/kg). The present study may promote utilization of forest litter as natural resource, thereby preventing the wild forest fires which destruct local ecology.

Keywords: Anaerobic digestion, biomethanation, lignocellulose, pine needles, renewable energy.

INDIAN energy scenario is presently facing an unprecedented energy crisis as the conventional energy resources of India are consistently deteriorating with limited stock of these natural minerals posing a staggering threat to the Indian economy. Among all the available resources, biomass seems a satisfactory substitute for compensating the energy void due to these natural resources[1]. Biomass is a photosynthetic biological material, hence, a renewable resource with almost zero net CO_2 emission that accounts for nearly 33% of a developing country's energy needs. In India, it meets about 75% of the energy needs of rural population which constitutes about 70% of the total population. Biomass refers to all biologically produced matter and all earth's living matter whose energy is derived from plant sources, such as agricultural, forestry, agro-industrial, human or animal wastes, which can be converted into convenient solid, liquid or gaseous fuels.

Fast growing forests with regular leaf shedding like chir pine litter have more capacity for carbon sequestration. Pine needles constitute lignocellulosic biomass which is still a predominant fuel being used for domestic purposes in the mid-altitude villages of the Indian Himalayas region (Uttarakhand, Himachal Pradesh and Jammu and Kashmir) which produces about 159 lakh tonnes of pine needles every year[1,2]. However, Uttarakhand Pine forests contribute about 8.23 lakh tonnes of surplus dry biomass each year[3,4].

Pine needles are a difficult forest waste. They cannot serve as fodder. They do not even decay like the other biomass and piled up pine needles are a major cause of wild forest fires. They are however, a good source of biomass fuel.

Additionally, dry pine foliage stops water from being absorbed by the soil and thus causes depletion of groundwater table[5]. Furthermore, fallen dry pine foliage acts like a carpet on the forest floor and blocks the sunshine reaching ground, stopping the growth of grasses which the cattle feed upon.

Every year forest fire causes great loss to the forest ecosystem, diversity of flora and fauna and economic wealth. Forest fires annihilate large tracts of virgin forests and cause irreparable damage[6]. In 1995, forest fire destroyed more than 3.75 million hectare of forest wealth, whereas in 1999, around 5085.6 sq. km and 1225 sq. km in 2008 were destroyed in Uttarakhand alone[7]. Hence, studies are undertaken to explore naturally fallen (*Pinus roxburghii*) foliage for generation of biofuels which is neither being used for human consumption nor for animal feed though it is a lignocellulosic biomass.

Biodegradation of lignocellulosic biomass

Though lignocellulosic biomass is a potential source of carbohydrate polymers for fermentation into simple sugars and subsequently to biofuel, saccharification of its structural polysaccharides into simple sugars is highly challenging due to its intrinsic recalcitrant nature of the

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lignocellulosic plant matter. It has been established that with increase in extracellular cellulase and xylanase production from hydrolytic microorganisms, biodegradation of pine needles increases[8]. Being rich in cellulose and hemicelluloses, pine needles can serve as a substrate for biodegradation and for the production of useful byproducts like biofuels besides its safe disposal helping to solve the burning issue of global warming. However, pretreatment is required to alter the biomass macroscopic and microscopic size and structure so that carbohydrate fractions to monomeric sugars can be achieved more rapidly and with greater yields that can be further fermented to biofuel[9].

Several pretreatment methods have been suggested for better degradation of lignocelluloses into fermentable sugars by various combinations including physical, physicochemical, chemical, and biological pretreatment to improve biogas production[10,11]. Among various physical pretreatments including hot water and steam-explosion mechanical/milling was shown to improve enzymatic hydrolysis by increasing the surface area of lignocellulosic materials[12–18]. Besides timber and resin, pine forests contribute to the agro-ecosystem in the form of plant nutrients, which are made available by the decomposition of pine litter. Being a coniferous litter rich in silica and phenolic compounds such as tannins, it is highly resistant to decomposition. Northup *et al.*[19] reported that they enter the soil affecting soil nutrient dynamics by delaying organic matter decomposition and mineralization. Studies on *Trichoderma reesei*, a mesophilic and filamentous fungus has gained significant industrial importance over the past decades due to its still yet unmatched hemicellulase producing capacities capable of plant cell wall degradation[20–23].

The bacterial additive is one of the methods for enhancing biogas production. Attar *et al.*[24] and Rabah *et al.*[25] studied the presence of *Bacillus megaterium*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus brovis*, *Bacillus alvei*, *Bacillus lentus*, *Yersinia nterocolitica*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella* sp., from the spent slurry during biogasification of abattoir waste. Rabah *et al.*[25] and Leonowicz *et al.*[26] studied the biodegradation of lignin by white-rot fungi related to the biochemistry of lignocellulose transformation. The ability of white-rot fungi to withstand and degrade tannin is well known[27]. *Trichoderma virescens* and *T. reesii* were able to tolerate up to 3000 ppm of pine needle crude tannins without any growth inhibition[20]. However, a much smaller group of filamentous fungi has evolved with the ability to break down lignin, the most recalcitrant component of plant cell walls. These are known as white-rot fungi, brown-rot fungi. Some soft-rot fungi can degrade lignin, because they erode the secondary cell wall and decrease the content of acid-insoluble material (Klason lignin) in angiosperm wood. Soft-rot fungi typically attack higher moisture, and lower lignin content materials[28]. Crystallinity of biologically pretreated lignocellulosic biomass of *Pinus densiflora* decreased by using enzymes from brown-rot fungi[29].

Chen *et al.*[30] reported the presence of *P. fluorescens* and *P. aeruginosa* in the biogas reactors that showed impact on nitrogen/nitrate ratio and removal of hydrogen sulphide and nitrate[30] *P. fluorescens* is a nonpathogenic Gram-negative, rod-shaped, oxidase positive, aerobic bacteria that inhabits soil, plants, and water surfaces with optimum growth temperature 25–30°C. Some of its strains possess many extracellular hydrolytic enzymes capable of degrading and using components of plant tissues such as hydrocarbon molecules, fatty acids and oils and can hydrolyse proteins[31]. Azin *et al.*[32] reported that being a saprophyte adapted to thrive in diverse situations, *Trichoderma* spp. produces a wide array of enzymes including chitinase. Labat *et al.*[33] studied the enzymatic hydrolysis of sugar beet pulps by *T. harzianum* cellulases for biogas production. They reported an average biogas yield of 0.67 m^3/kg VS with production rate of 0.4 m^3/m^3 of digester per day with an average methane content of 58%.

However, pine needles biomass can be converted to useful renewable fuels such as biogas, biooil, producer gas and briquettes using appropriate physical, thermochemical or biochemical conversion processes. This may protect the natural resources, flora and fauna of the region and provide rural livelihood.

In view of the availability of large quantities of pine needles as forest wastes and its vast energy potential, a study was undertaken to assess the biodegradation of inert lignocellulosic pine needles for biogas production by a group of bioagents consisting of *P. fluorescens* and *T. harzianum*.

A laboratory-scale, batch type anaerobic digester was used to evaluate the variation in process parameters, biological methane potential of pine needles during anaerobic digestion and their effects on biodegradability.

Experimental procedure

The experimental set-up used to carry out biomethanation of pine needle substrates consisted of a batch type fermenter of 4 litre capacity polymer reagent bottle, gas transfer tube and two litre capacity gas collection bottle as shown in Figure 1. All the joints were sealed with synthetic rubber adhesive and M-seal and soap solution was applied for detecting any gas leakage.

Collection of raw materials

The substrates for the experiments, pine needles, inoculum and culture for biotreatment were collected from

University research centres. The pine needles were collected from the Institute of Biotechnology, Patwadanger, Nainital. The *Trichoderma* spp. and *Pseudomonas* spp. culture were procured from the Biocontrol Laboratory, Department of Plant Pathology, whereas the inoculum from a 2 m^3 size Pant RCC model biogas plant operating on dairy cattle waste was collected from the Bioenergy Field Research Laboratory.

Preparation of substrates

The substrates used for anaerobic batch fermentation were naturally fallen pine needles with or without biotreatment. The naturally fallen collected pine litter had total solids of about 85%. The procured pine needles were sun-dried before storage and its size was reduced by grinding to an average mesh size of about 2 mm (refs 14, 15).

Analysis of pine needles shows that it has calorific value of 4690 k cal/kg, pH 6.1 and volatile content 72.4%, hemicellulose 11.4%, cellulose 18.2%, lignin 9.1%, organic carbon 34%, TKN 0.73%, C/N ratio 46.6%, and ash content 2.5% (refs 34, 35).

The biotreated substrate (T_2) was prepared by thoroughly mixing 2 g culture of *Trichoderma* spp. and *Pseudomonas* spp. with ground pine needles. Both the substrates were saturated with distilled water and kept in PVC bags for 15 days to increase its solubility[30–33]. Thereafter, the substrates were prepared by mixing with 30% active seed inoculum[36–38] (Table 1).

A blank treatment (inoculum without pine needle) was also tested with three replications to compensate biogas yield due to inoculum alone. The total biogas production in the experiment was evaluated after deducing the gas production due to inoculum.

The experiments were carried out with 2 kg of substrate for each fermenter and placed in a thermostatically controlled hot air oven maintained at 35 ± 1°C. The gas transfer tube from each fermenter was taken out from oven and connected to the gas collection bottle filled with brine solution. The daily biogas production was measured volumetrically by observing displaced brine solution into measuring cylinder.

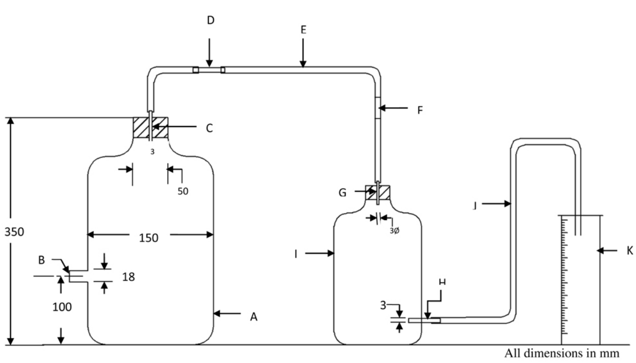

Figure 1. Experimental set-up: [A] fermenter, [B] sampling port, [C] copper tube for gas outlet, [D, F, G, H] copper tube jointer, [E, J] rubber tube, [I] gas collection bottle, [K] measuring cylinder.

Determination of TS, VS, pH and total alkalinity

A sample from each fermenter was drawn after every seventh day to determine the total solids (TS), volatile solids (VS) and total alkalinity available in substrates according to Standard Methods for the Examination of Water and Waste Water[39,40]. The samples for TS, VS, pH and total alkalinity were analysed initially, weekly and at the termination of experiment.

Analytical methods

The TVS removal efficiency was expressed as the percentage removal of initial total volatile solids during the week. It is determined by the equation

$$\text{TVSRE \%} = (\text{TVSMR}/\text{TVSI}) \times 100,$$

where TVSMR is the periodic total volatile mass removed and TVSI is the total initial, volatile mass (g). The biodegradability of pine needle substrates at the termination of experiment was evaluated using

$$\text{Biodegradation \%} = (\text{TVSI} - \text{TVSF})/\text{TVSI} \times 100,$$

where TVSI is total initial volatile solids and TVSF is the final total volatile solids (g).

Determination of biogas production

The biogas was produced at 35 ± 1°C but collected at NTP. In order to evaluate the volume of biogas produced at STP, it was corrected to exclude the volume occupied by water vapour according to Charles' law[41]. The corrected volume of biogas at STP was obtained by multiplying it with corresponding dry biogas factor (DBF) expressed as

$$\text{dry biogas factor (DBF)} = 0.99998 - 0.004501 \times T,$$

where T is the ambient temperature (°C). The methane yield was obtained by multiplying dry biogas volume at STP with the corresponding methane content as analysed by gas chromatograph.

Biogas analysis

The gas composition (methane content) was determined using plunger displacement of an air tight syringe using

Table 1. Values of different ingredients for preparation of substrates

Types of substrate	Weight of substrate pine (kg)	Weight of water added (kg)	Weight of inoculum added (kg)	Initial TS of substrate (%)	Initial VS of substrate (%)
Untreated pine needle T_1	0.380	1.02	0.600	18.48	15.97
Bio-treated pine needle T_2	0.380	1.02	0.600	16.46	14.46

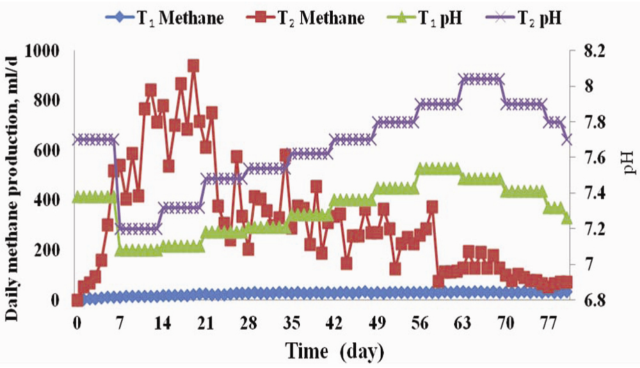

Figure 2. Effect of pH on dry biomethane production of pine needle substrates at STP.

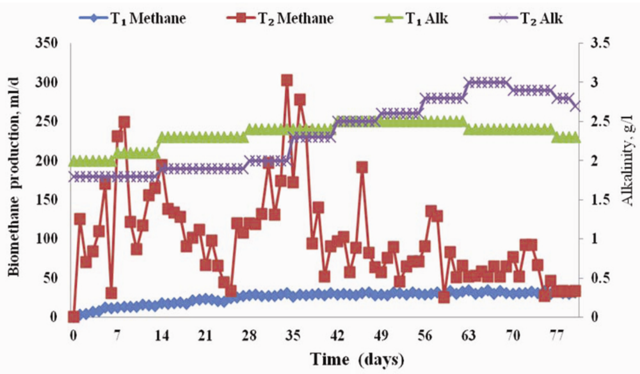

Figure 3. Effect of total alkalinity on dry biomethane production of pine needle substrates at STP.

Nucon make gas chromatograph having thermal conductivity detector with Porapak Q packed column, standard methane sample and helium as carrier gas with gas flow rate of 60 ml/min.

Discussion

Anaerobic treatment process is recognized as a simple and energy-efficient way of treating and stabilizing different types of wastes. The process uses relatively simple equipment to directly produce usual biofuel and finds application in a variety of industries.

The experiment shows that biodegradation of substrates started slowly and proceeded without any problem. Experimental results of various parameters pH, total alkalinity, TS and VS, are represented in Figures 2–5 respectively, and discussed below.

pH

pH plays an important role in microbial activities during anaerobic biodegradation. A decrease in pH was observed during the first week due to formation of volatile fatty acids (VFA) during acidification stage due to activity of acetogenic bacteria (Figure 2)[42–44]. The pH drop inhibits the initiation of biomethane production process causing lesser biogas production due to the acidic nature of substrate[45].

Thereafter, the gradual increase in pH was due to the conversion of VFA into biogas due to action of methanogenic bacteria. An increasing trend of biomethane production was also observed with increase in pH and attained a maximum value of 303 ml for treated (T_2) whereas only 30.5 ml on 34th day with observed pH values of 7.49 and 7.28 for treatment T_2 and T_1 respectively.

Alkalinity

Total alkalinity provides the required buffering capacity which acts as a safeguard against pH fluctuations due to volatile acids. Therefore moderate total alkalinity should be maintained for the stability of anaerobic biodegradation system[46,47]. The alkalinity as analysed during the study was in the range of 1.9–3 and 2–2.5 g/l for untreated and biotreated substrates respectively, which was within the required limit (Figure 3)[46,47].

The alkalinity was found to vary proportionately like pH values and shows its impact on biomethane production rate with gradual increase in biomethane yield with a value of 303 ml and 30.5 on 34th day with total alkalinity of 2.3 and 2.4 g/l for treatment T_2 and T_1 respectively (Figure 3).

Total solids and volatile solids

The variation in TS and VS values during anaerobic biodegradation of pine needles substrates is shown in Figures 4 and 5 respectively. The variations in average TS of each fermenter were observed in the range of 372.37 to 356.67 g for untreated (T_1) and 329.2 to 306 g for biotreated substrate (T_2) having initial TS of 18.41% and 16.41% respectively.

However, the variations in VS were found in the range of 321.8 to 306.42 g for T_1 and 289.2 to 259.1 g for T_2 having initial VS of 15.97% and 14.46% respectively.

The average reduction in TS was found to be 4.21% and 7.04%, whereas it was 4.78% and 10.41% for VS for treatment T_1 and T_2 respectively (Table 2).

TVS removal and biodegradability

Anaerobic digestion of organic wastes leads to the reduction in TS and VS. The variation in total volatile solids removal efficiency (TVSRE) during anaerobic fermentation of substrates is shown in Figure 6. Results indicate that pine substrate has a biodegradability of 4.78% and 10.41% in 80 days of fermentation period which is comparable to the findings of Misi and Forster[48,49]. A higher TVS removal during second week might be due to availability and utilization of more volatile suspended solids by microorganisms during initiation process. TVSRE is also an indicator of gas yield, it follows similar trends as observed in daily biogas production as evident from Figure 6 though, it is also governed by variation in other process parameters[50].

Biogas production

The variation in daily biogas production of substrates with pH alkalinity and VS, during anaerobic batch fermentation is represented in Figures 2, 3 and 6. It was observed from experimental results that biogas yield increased with increase in days of operation and found to vary with reduction of total volatile solids content in the fermenter[51–53]. Acidogenic bacteria at an early stage of digestion produce organic acids (VFA) quickly which lowers the pH as shown in Figure 2. Hence, biomethane production decreases till 12th day. The methanogenic bacteria take longer time to acclimatize and consume VFA at this lower pH. A gradual increase in biogas yield was observed after 4th week and it approaches from 175.7 to 430.2 ml d^{-1} till the end of 5th week for biotreated and from 51.2 to 54.7 ml d^{-1} for untreated substrate during the same period (Figure 6)[54]. However, there was a gradual increase in biomethane production rate after 2nd week (27.80 and 30.56 ml to 45.4 and 264.1 ml) up to the end of 5th week from the above treatments. Thereafter, a decrease in biomethane yield was observed due to reduction in availability of biodegradable volatile solids. Results indicate a cumulative biomethane yield of 2.04 and 8.10 liters from 2 kg substrate from 380 g pine in each fermenter with specific biogas yield of 0.247 and 0.459 lg^{-1} VS destroyed for untreated and biotreated

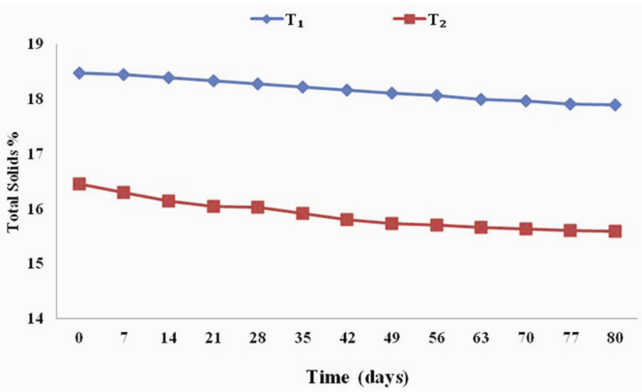

Figure 4. Variation TS of pine needle substrates with time.

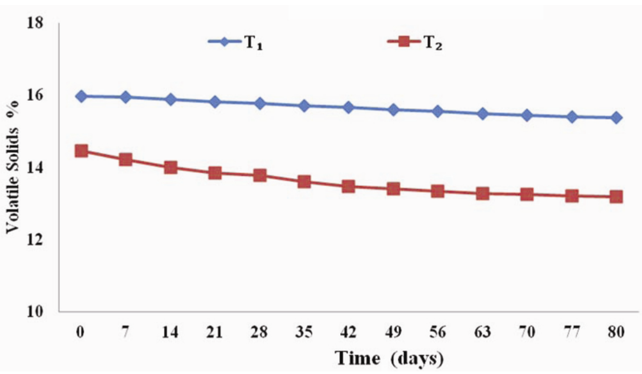

Figure 5. Variation VS of pine needle substrates with time.

Table 2. Performance of pine needle substrates during anaerobic batch fermentation at 35 ± 1°C

Parameters	Treatments T_1	T_2
Reduction in TS (g)	15.70	23.19
Reduction in TS (%)	4.21	7.04
Reduction in VS (g)	15.37	30.1
Biodegradability (%)	4.78	10.41
Biogas yield (l)	3.81	13.83
CH_4 yield (l)	2.04	8.10
Biogas yield l/g TS destroyed	0.242	0.596
Specific biogas yield l/g VS destroyed	0.247	0.459
methane yield l/g VS destroyed	0.133	0.269
methane yield l/kg pine needle	5.53	21.30

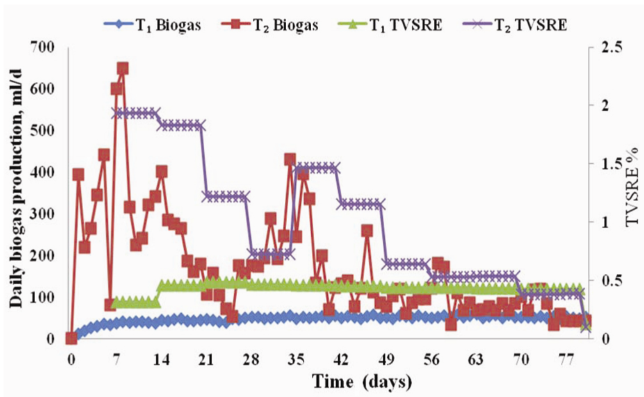

Figure 6. Relationship between TVSRE with biogas production at STP 35 ± 1°C.

substrates respectively. However for conventional cow-dung substrate it has been reported as 0.76 lg^{-1} VS (refs 50, 55).

The present study on *P. roxburghii* leaves shows their potential for biodegradation with high prospects for further conversion to biomethane by employing suitable pretreatments and exploiting their use as a substrate for cellulose and xylanase production. The results are similar to those reported earlier for NaOH treated waste pine[56].

Biogas technology has established itself and could exercise major influence in the energy scenario in rural India despite the presence of about only 47 lakh biogas plants against installed potential of 120 lakhs[57].

The foregoing study reveals that biotreated pine needle substrate has the biogasification potential of about 36.3 l/kg of pine needles. Considering 40% availability of pine needles for biogasification in Uttarakhand, 2.98×10^{10} m^3 of annual biogas can be produced from 8.23 lakh tonne of surplus[4] annual pine needles which might be equivalent to 2.98×10^{10} kWh annual electricity production.

Conclusions

This study suggests the eco-friendly solutions for degradations of highly inert fallen foliage like pine needles without compromising with the land use and human/animal feed. The physico-biological pretreatment of pine needles by *T. harzianum* and *P. fluorescens* is effective for its biomethanation. It was found that a biomethane yield of 21.3 l/kg pine needles can be obtained from pretreated pine needles substrate which was 300% higher compared to untreated pine needles substrate. By the analysis of foregoing results and parameters governing the anaerobic biodegradation process, it can be concluded that pine needles litter is feasible and has enough potential for biomethane yield as a green fuel.

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