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Development of anaerobic consortia and its invitro evaluation for biomethanation potential of coffee processing wastes

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An anaerobic consortia was prepared by using *Clostridium* strains already screened for biocatalytic ability to degrade cellulose, pectin and tannins together with *Methanosarcina* sp. Among the two inoculum sources studied, the reactor having anaerobic consortium could ferment coffee pulp waste (CPW) and coffee processing waste water (CPWW) leading to 67% soild removal, 62.27% pectin, 27.4% tannins, 63.52% cellulose and 60% hemicellulose reduction. This resulted in total biogas production of 0.017 m^3 over a period of eight weeks and highest methane content of 65% at 6^{th} week when compared to cow-dung slurry. The bioenergy obtained was converted into electricity units saving 27.2% of power consumption with anaerobic consortium for a small scale coffee processing unit generating 5 ton of coffee pulp waste. This study indicates that anaerobic consortium with efficient microbial strains appeared to be a promising technology for mitigating the present problems caused by coffee processing wastes.

Key words: Anaerobic consortium, biogas, methane, total solids (TS) removal, biopolymers.

INTRODUCTION

Anaerobic biological system offers the greatest potential for the treatment of industrial effluent. Anaerobic decomposition of complex organic matter into methane and carbon dioxide is a complex process, involving a well-organised community of several microbial populations. The flow pattern and the formation of intermediate metabolites during degradation depend on the microbial status and the operating conditions. Biogas production through anaerobic digestion of biomass including the organic fraction of waste materials and residues is a particularly promising choice and experiences increasing interest worldwide (Onodera et al., 2012). The anaerobic degradation of organic polymers is a multistage process such as hydrolysis, acidogenesis, acetogenesis and methanogenesis. These processes do not have high energy demand resulting in low biomass production and generation of methane, which can be used as an energy source (Ma and Ong, 1988).

Coffee processing industries emanate huge volumes of wastes in the form of coffee pulp and coffee processing waste water. Coffee pulp waste containing sizable proportion of compounds like pectin (6.5%), reducing sugars (12.4%), non reducing sugars (2.0%), caffeine (2.3%), chlorogenic acid (2.6%), lignin, cellulose etc. could be digested anaerobically to produce biogas. Coffee processing waste water (CPWW) is loaded with high organic matter rather than its inherent toxicity. The major constituents of CPWW are suspended solids and

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Abbreviations: CPW, coffee pulp waste; **CPWW,** coffee processing waste water; **AC,** anaerobic consortium; **CD,** cowdung slurry.

dissolved solids containing pectin, proteins and sugars which are biodegradable in nature. The concentrations of these pollutants vary with the quantity of water used for processing of fruits (Shanmukhappa et al., 1998). During the fermentation process in wastewater, the acidification of sugars will drop the pH to around 4 or less, and the digested mucilage will be precipitated out of solution and will build a thick crust on the surface of the wastewater. At around pH 7 and over, flavanoids turn wastewater into dark green to black colour staining rivers downstream from coffee factories. Considering the serious impact of coffee processing wastes to the environment, anaerobic digestion holds an alternate low cost energy conserving mitigation.

The total solids and the volatile solids have been reduced considerably during the anaerobic digestion of coffee pulp. Methane production from coffee wastes has been enhanced by the addition of cowdung and old slurry of a biogas plant. The potential daily yield of biogas from 2 ton of coffee pulp is approximately 131 m^3 equivalent in terms of its fuel value to 100 lts of petrol (Houbron et al., 2003). Maximization of biogas production has also been undertaken (Boopathy and Mariappan, 1984) when coffee waste, cowdung and old slurry were mixed in the ratio of 3:1:1. Efficient treatment of coffee processing waste water employing cowdung slurry as inoculum have been reported by Selvamurugan et al. (2010).

Cellulolytic bacteria and Methanogenic Archaae represents one of the components of cowdung slurry or old digested slurry or rumen fluid. Screening of efficient strains from these sources can be effectively utilized for sustainable degradation of lignocellulosic biomass degradation and thereby biofuel generation. Till date only cowdung slurry, digester slurry or goat rumen is widely employed as inoculum source for anerobic digestion of coffee wastes.

A definite paucity of information exists regarding the pectinolytic behavior of cellulolytic *Clostridium* sp and syntrophic association of *Clostridium* sp with Methanogenic archae. Our efforts would be directed towards developing anerobic consortia using cowdung slurry as matrices and co-inoculating with well characterized *Clostridium* sp involved in biodegradation of polymers and methanogenic archae as partners in the association.

MATERIALS AND METHODS

Growth and maintenance of the strains

A set of five anaerobic isolates and five methanogenic archae were isolated from coffee processing wastes by enrichment technique using goat rumen fluid. The enrichment technique was used to enhance the population of anaerobic bacteria involved in bioconversion, since the native population might be low in the coffee wastes. The coffee pulp and coffee processing waste water were mixed with goat rumen fluid in equal proportion under anaerobic condition and incubated at 37°C for two weeks. Anaerobic bacteria were isolated by Hungate's roll tube technique using specific selective media. The anaerobic isolates were screened for their biodegradation potential of complex biopolymers vis., cellulose, pectin, tannic acid, and hemicelluloses represented by coffee processing wastes. The elite strains TCW 3 and TCW 5 for efficient biodegradation belonging to the genus *Clostridium* were maintained in modified Hungates's medium (Ramasamy et al., 1992). The exponential phase cultures (12-15 days), incubated at 35+/- 2°C were used for further studies.

The methanogenic archae was maintained in Mah medium with acetate (Mah et al., 1978) and were screened based on their ability for methanogenesis and methane recovery. The selected strain *Methanosarcina* sp. (TCWMS 5) was used after repeated subculturing in Mah medium and was grown under anaerobic condition at 35+/- 2°C.

Co-cultivation of Clostridium and Methanosarcina sp.

To a two welled anaerobic glass container, 50 ml of sterile CPWW and 25 g of sterile CPW was transferred to the main well and 10 ml of sterile CPWW and 5 g of sterile CPW was transferred to the test tube well. The two mouths of the glass container were sealed with a butyl rubber stopper and aluminium cap.

The contents were sterilized at 121°C for 10 min and allowed to cool. The main well was inoculated with 5 ml of fresh cells (10^6/ ml) of *Clostridium* sp as per the experimental details mentioned below through disposable needles and syringes in N_2 atmosphere. In the test tube well, 2 ml of *Methanosarcina* sp. (TCWMS 5) was inoculated and incubated at 37°C.

The methane content of the gas phase in the anaerobic glass container was measured after the third day by KOH displacement method using saccharometer. Residual cellulose, pectin and tannic acid were also assessed using standard procedures.

Arrangement of experiment for co-culture studies

The arrangement of experiment for co-culture studies included: T_1, *Clostridium* sp. TCW 3 + *Methanosarcina* sp. TCWMS 5 + SCPW +SCPWW; T_2, *Clostridum* sp. TCW 3 + *Methanosarcina* sp. TCWMS 5 + SCPW +SCPWW**;** T_3, *Clostridium* sp. TCW 3 alone in the main well + SCPW +SCPWW**;** T_4, *Clostridium* sp. TCW 3 alone in the main well + SCPW +SCPWW; T_5, *Methanosarcina* sp. TCWMS 5 alone in the test tube well + SCPW +SCPWW; T_6, both wells uninoculated + SCPW +SCPWW

Development of anaerobic consortia

The strains *Clostridium* sp. (TCW 3 and TCW 5) and *Methanosarcina* sp. (TCWMS 5) were mass multiplied in their respective media. Optimization of inoculum rate of the three partners was undertaken by using 2-10% anaerobic cultures vis a vis 2-10% of methanogenic archae in sterile cowdung slurry as a matrice for multiplication.

After one week of growth under optimum conditions, samples were drawn periodically at weekly interval and examined for their population. Microscopic analysis was carried out to study the survival and colonization All the cultures inoculated at the rate of 10% was found to be optimum showing maximum number of colonies.

Invitro evaluation of anaerobic consortia in bench scale fermentation of coffee wastes

A bench scale anaerobic fermentation experiment was set up to assess the performance of anaerobic consortia interms of biocatalysis and methanogenesis using coffee wastes viz., CPW and CPWWW as substrates. The coffee pulp was made into slurry

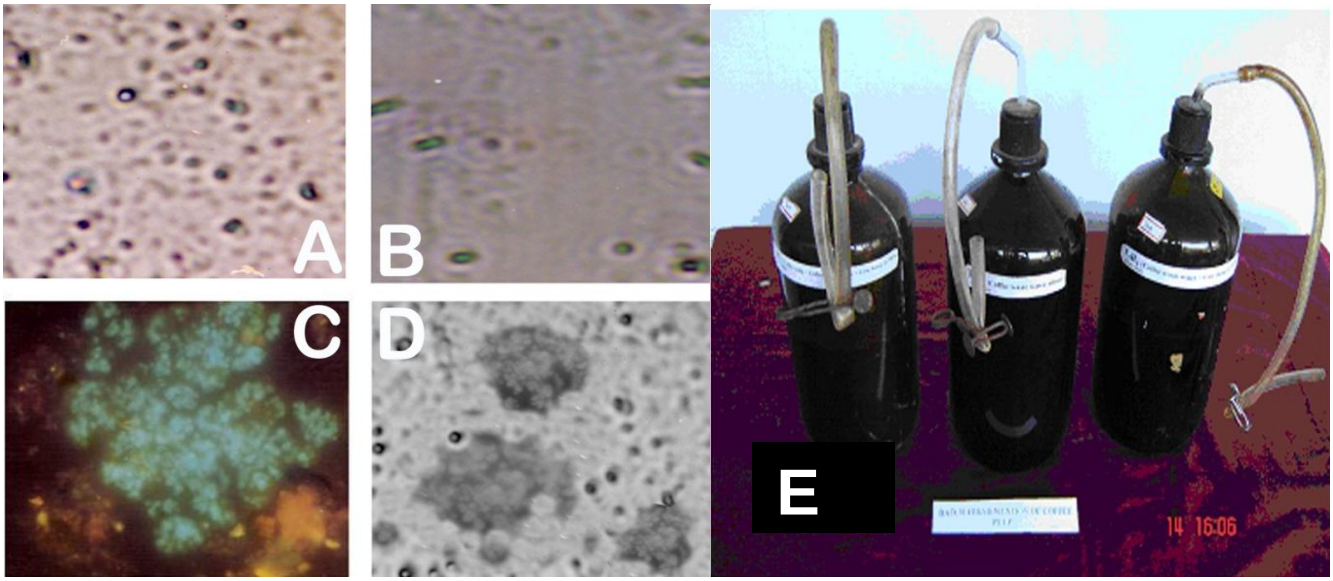

Figure 1. A, *Clostridium* sp. TCW 3; **B,** *Clostridium* sp. TCW 5; **C,** *Methanosarcina* sp. **D,** Microscopic view of anaerobic consortium. E, Experimental set up for bench scale anaerobic fermentation of coffee processing wastes

with coffee processing waste water (250 g of CPW and 1000 ml of CPWW)
so as to have a TS per cent of 25%. Amber colored glass containers of 2.5 L capacity were filled with coffee pulp slurry as per the experimental details given below. The performance of anaerobic consortia was compared with cowdung slurry from an active cow dung based plant. Both the inoculums were used at a rate of 10 per cent of the substrate volume. Only 2/3 of the bottles were filled with slurry and inoculum and 1/3 of the space was allowed for biogas collection (Figure 1E).

An assembly of bent glass tube fixed tightly on rubber cork and attached to rubber tubes with a pinch cork was used to create airtight condition. Each treatment was replicated thrice and the biogas generated was recorded daily by water displacement method and the methane content was quantified in a Gas Chromatograph with thermal conductivity detector (TCD) having 'Porapak Q' column by setting the oven temperature at 80 to 100°C, injector temperature at 100 to 200°C, detector temperature at 120°C and using nitrogen as carrier gas at a flow rate of 30 ml min^{-1}.The change in total solids, pH, EC and the degradation of biopolymers such as pectin, cellulose, hemi cellulose and protein were assessed at periodic intervals (APHA, 1992).

Experimental set-up for bench scale anaerobic fermentation

Experimental set-up for bench scale anaerobic fermentation included: T_1, Coffee pulp alone (CPW); T_2, Coffee waste water alone (CWW);T_3, CPW + CWW; T_4, CPW + cow dung slurry (10%); T_5, CWW + cow dung slurry (10%); T_6, CPW + CWW + cow dung slurry (10%); T_7, CPW + Anaerobic consortia (10%); T_8, CWW + Anaerobic consortia (10%); T_9, CPW + CWW + Anaerobic consortia (10%)

Statistical analysis

The data recorded in triplicates for the growth and biochemical parameters in selected treatments were subjected to ANOVA (Analysis of Variance) in accordance with the experimental design (completely randomize block design) using SPSS.10 statistical package were used to quantify and evaluate the source of variation.

RESULTS

A set of five anaerobic strains isolated from coffee processing wastes by enrichment technique were screened in terms of biocatalysis and growth on different substrates viz., cellulose, pectin and tannin. Two strains identified as *Clostridium* sp (TCW 3 and TCW 5) exhibited more efficiency in biodegrading cellulose and pectin, tannic acid respectively on 12th day. The combination of both strains performed significantly in terms of percent reduction of cellulose, pectin and tannic acid rather than individual strains (data not shown). A gradual increase in VFA like acetate upto 12 days, and propionate upto six days was observed, however VFA declined thereafter. The five archael strains were screened based on cumulative biogas production and methane generation. The strain TCWMS 5 reported significant biogas production ability and methane recovery. The microscopic analysis confirmed TCWMS 5 as well distinct sarcina type of cells (Figure 1C).

Co-culturing of *Clostridium* sp. and *Methanosarcina* sp.

Considerable work has been available on the cellulolytic behavior of *Clostridium* sp but its quiet rare to obtain a *Clostridium* strain with the ability to degrade pectin and tannins in addition to cellulose. Co-culturing of *Clostridium* sp. and *Methanosarcina* sp. resulted in significant reduction of pectin content. On the 8th week, maximum

Table 1. Per cent reduction of biopolymers on 8th week of coculturing.

Treatment	Pectin (%)	Tannin (%)	Cellulose (%)
T_1	42.62	28.95	56.35
T_2	54.1	47.37	44.44
T_3	31.15	23.68	47.09
T_4	36.07	36.81	37.83
T_5	14.75	5.26	6.08
T_6	8.19	7.89	3.44
SEd	0.095	0.099	0.123
CD(0.05)	0.208	0.217	0.267

T_1, TCW 3 + TCW MS 5 + SCPW + SCPWW; T2, TCW 5 + TCW MS 5 + SCPW + SCPWW; T_3, TCW 3 alone in the main well + SCPW +SCPWW ;T_4, TCW 5 alone in the main well + SCPW +SCPWW ; T_5, TCW MS 5 alone in the test tube well + SCPW + SCPWW; T_6, Both wells uninoculated + SCPW +SCPWW. SCPW, sterilized coffee pulp waste; SCPWW, sterilized coffee processing waste water. *Values are average of three replications.

Table 2. Cumulative biogas production during co-culturing.

Treatment/ week	Cumulative biogas production (ml/week)								
	I	II	III	IV	V	VI	VII	VIII	Total
T_1	45	80	120	185	220	245	230	185	1310
T_2	55	85	105	158	196	242	215	165	1221
T_3	-	-	20	35	55	40	-	-	150
T_4	-	-	25	40	65	50	-	-	180
T_5	-	-	55	88	105	125	85	65	523
T_6	-	-	-	15	25	40	35	20	135

T_1, TCW 3 + TCW MS 5 + SCPW + SCPWW; T2, TCW 5 + TCW MS 5 + SCPW + SCPWW; T_3, TCW 3 alone in the main well + SCPW +SCPWW; T4, TCW 5 alone in the main well + SCPW +SCPWW ; T_5, TCW MS 5 alone in the test tube well + SCPW + SCPWW; T_6, Both wells uninoculated + SCPW +SCPWW. SCPW, Sterilized coffee pulp waste; SCPWW, sterilized coffee processing waste water. *Values are average of three replications.

reduction in pectin content (54.1%) was observed with *Clostridium* sp. TCW 5 and *Methanosarcina* sp. (TCWMS 5) followed by *Clostridium* sp. TCW 3 and *Methanosarcina* sp. (TCWMS 5). The tannic acid content reduced towards the period of incubation and the reduction per cent varied significantly among all the treatments. Co- culturing of *Clostridium* sp. TCW 5 and *Methanosarcina* sp. (TCWMS 5) recorded maximum reduction of tannic acid (47.37 per cent) followed by *Clostridium* sp. TCW 5 alone. For cellulose degradation, *Clostridium* sp. TCW 3 with *Methanosarcina* sp. (TCWMS 5) recorded maximum reduction of cellulose (56.35%) followed by *Clostridium* sp. TCW 3 alone (47.09%). The results are presented in Table 1.

Co-culturing and Methanogenesis

During co-culturing, an increasing trend of biogas production was noticed upto the 6th weeks in all the treatments (Table 2). Maximum biogas production was recorded by *Clostridium* sp. TCW 3 and *Methanosarcina* sp. TCWMS 5 (1,310 ml) followed by *Clostridium* sp. TCW5 and *Methanosarcina* sp. TCWMS 5 (1,221 ml). The results opened new platform of bringing the two strains of Clostridium sp with the ability to degrade complex polymers viz., cellulose, hemicelluloses, pectin tannins and *Methanosarcina* sp. which could increase the efficiency of anaerobic fermentation.

Development of anaerobic consortium and its survival studies

The population of the inoculated strains *Clostridium* sp and *Methanosarcina* sp. was enumerated in their respective media before inoculation and monitored to evaluate the viability of the cells. Despite a 10-25% reduction in the number of cells after one week, the cells remained static till 3 weeks and declined later (Figure 2). The bacterial cultures showed a similar level of reduction with time. Protein accumulation also showed a drastic reduction after 3 weeks (data not shown). The viability and survival of the strains in the consortium were also confirmed by microscopic analysis (Figure 1A, B, C and D).

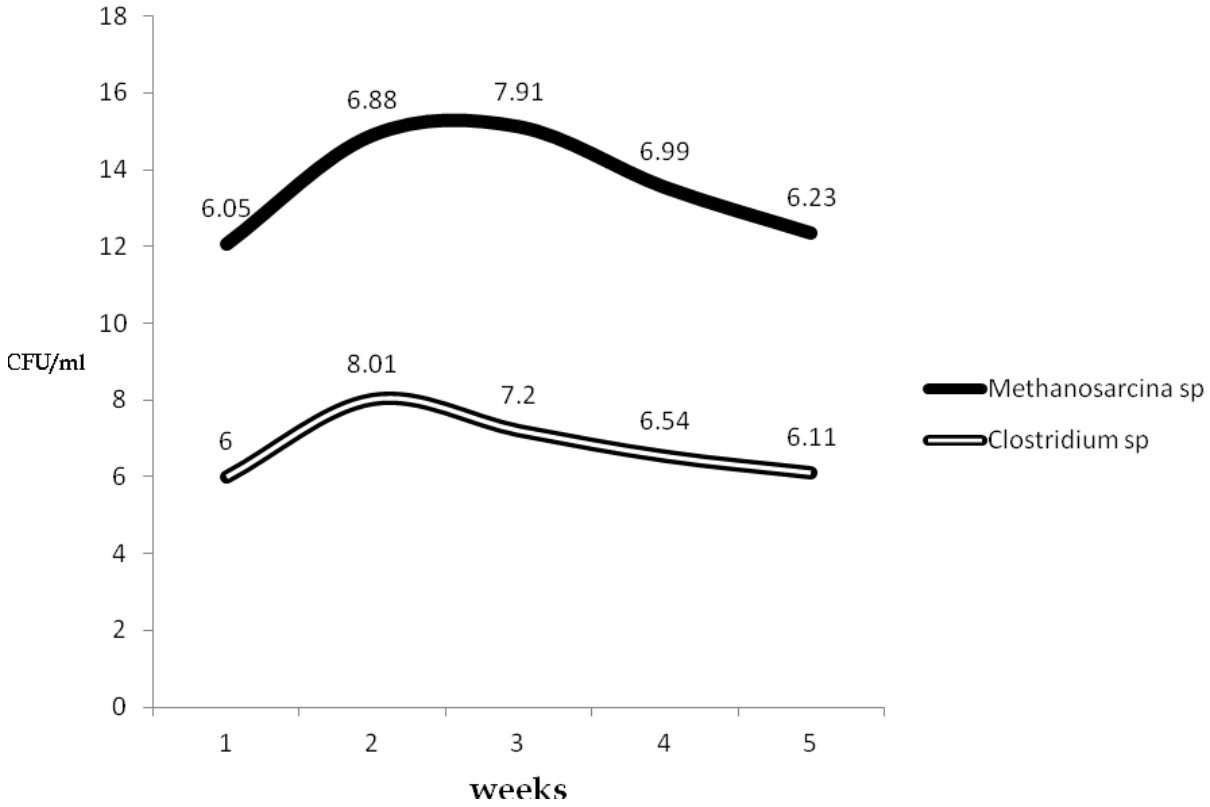

Figure 2. The log CFU/ml of inoculated strains at different intervals.

Invitro evaluation of anaerobic consortium for anaerobic fermentation of coffee processing wastes

Effect of anaerobic consortium on pH and EC

The anaerobic fermentation process is known to be extremely sensitive to pH. During the course of the study, pH gradually decreased on 30th day and increased on 60th day irrespective of all the treatments. The decrease in pH during fermentation may be due to the production of volatile fatty acids and later the pH increased because of methanogenic activity (Table 3). Data showed that EC declined gradually during anaerobic fermentation and maximum reduction was observed in treatments with 10% anaerobic consortium as inoculums rather than cowdung slurry 10%. This represents a good operation condition of the system which favours hydrolysis, acidogenesis followed by acetogenesis and methanogenesis.

Effect of anaerobic consortium on solid removal

The total solids (TS) have been reduced considerably during anaerobic fermentation of coffee pulp waste. In reactor with anaerobic consortium as inoculum source, on 60th day, the TS of the waste water and coffee pulp waste were 7.1 ^{1} and 90.5 g L^{-1} respectively. A maximum TS removal of 67% was achieved by CPW + CPWW + anaerobic consortium (10%). Solid removal efficiency was very poor in the reactors without any inoculum source. In reactor with cowdung slurry as inoculum the TS were 9.2 and 113.5 g L^{-1} respectively for coffee waste water and coffee pulp waste on 60th day. The TS removal efficiency with cowdung slurry was only 59.68%. At each stage, the total solids decreased and total biogas production increased due to faster metabolic rate of the organisms.

Effect of anaerobic consortium on biopolymers pectin, tannin, cellulose, hemicellulose

The biopolymers like pectin, tannic acid, cellulose, hemicellulose, proteins and reducing sugars were significantly reduced during anaerobic fermentation of coffee pulp waste. In the reactor with anaerobic consortium, pectin and tannic acid content were reduced by 62.27 and 27.94% respectively (Table 4). The per cent reduction of cellulose and hemicellulose were 63.52 and 60% respectively. But with cowdung slurry, only 51.25, 15.94, 47.44 and 45.85% reduction in pectin, tannin, cellulose and hemicellulose respectively was observed.

Table 3. Change in pH and EC during bench scale anaerobic fermentation of coffee processing wastes.

Treatment	pH			EC (dSm^{-1})		
	Initial	30th day	60th day	Initial	30th day	60th day
T_1 : Coffee pulp waste alone (CPW)	6.01	5.86	6.22	0.78	0.73	0.66
T_2 : Coffee waste water alone (CPWW)	4.50	4.41	4.63	0.98	0.95	0.91
T_3 : CPW + CPWW	6.33	6.06	6.52	1.5	1.2	1.0
T_4 : CPW + CD (10%)	6.54	6.07	7.03	1.3	1.1	0.95
T_5 : CPWW + CD (10%)	4.87	4.51	5.15	1.2	1.1	0.93
T_6 : CPW + CPWW + CD (10%)	6.66	6.44	7.11	1.8	1.3	0.90
T_7 : CPW + AC (10%)	6.52	6.10	7.18	1.7	1.3	0.85
T_8 : CPWW + AC (10%)	4.91	4.64	5.25	1.1	0.89	0.78
T_9 : CPW + CPWW + AC (10%)	6.73	6.34	7.23	2.1	1.1	0.70

Table 4. Per cent reduction of biopolymers during bench scale anaerobic fermentation of coffee processing wastes.

Treatment	% reduction in biopolymers on 60th day						
	Total solid	Pectin	Tannin	Cellulose	Hemicellulose	Protein	Reducing sugar
T_1 : Coffee pulp waste alone (CPW)	9.96	11.15	3.90	13.60	16.39	7.21	12.40
T_2 : Coffee waste water alone (CPWW)	8.44	17.20	5.68	14.44	8.04	8.57	6.10
T_3 : CPW + CPWW	13.8	14.08	5.86	20.77	16.81	11.51	12.60
T_4 : CPW + CD (10%)	54.56	44.29	11.11	42.00	38.79	42.95	36.40
T_5 : CPWW + CD (10%)	46.51	43.07	10.90	46.22	38.46	45.41	30.00
T_6 : CPW + CPWW + CD (10%)	59.68	51.25	15.94	47.44	45.85	53.55	42.20
T_7 : CPW + AC (10%)	63.80	58.64	21.21	59.87	57.73	55.63	50.00
T_8 : CPWW + AC (10%)	61.20	54.74	18.95	57.14	58.82	54.80	54.20
T_9 : CPW + CPWW + AC (10%)	67.00	62.27	27.94	63.52	60.00	62.88	57.20
SED	0.3080	0.0702	0.0860	0.0893	0.0755	0.0812	0.0159
CD (0.05%)	0.6471	0.1476	0.1806	0.1876	0.1586	0.1706	0.0333

Effect of anaerobic consortium on protein and reducing sugars

The protein content was found to decrease gradually during anaerobic digestion of coffee processing waste and maximum reduction of protein content (62.88%) was observed in reactor with CPW + CPWW and anaerobic consortium (10 %) followed by reactor with CPW and anaerobic consortium (55.63 %) on 60th day of fermentation. Reducing sugar content in all treatments increased during the first phase of fermentation and thereafter decreased. In the initial phase, the level of reducing sugar in the treatments ranged from 0.33 to 1.45%. At 30th day of anaerobic fermentation, all the treatments showed increased level of reducing sugars but at 60th day, significant reduction was observed in all the treatments. Maximum reduction of 57.2% was observed in the reactor with CPW + CPWW and anaerobic consortium 10% (Table 4).

Effect of anaerobic consortium on methanogenesis and methane recovery

As the fermentation proceeds, the volume of biogas and methane produced also increased. The performance of the reactor with anaerobic consortium was much better than cowdung slurry, on 6th week with maximum TS reduction of 67 % and the average biogas production was 5.5 m^3 (Figure 4). The methane content was 65% on 6th week and declined gradually. This increasing trend was noticed up to sixth week in reactors with 10% anaerobic consortium as inoculum and up to seventh week in reactors with 10% cow-dung slurry.

Efficiency of anaerobic fermentation of coffee processing wastes

The efficiency of anaerobic fermentation of coffee proces-

Table 5. Efficiency of bench scale anaerobic fermentation of coffee processing wastes.

Treatment	Total gas (l)	Biogas/gTS added (l/g)
T_1 : Coffee pulp waste alone (CPW)	2.16	0.087
T_2 : Coffee waste water alone (CPWW)	1.24	0.805
T_3 : CPW + CPWW	3.65	0.146
T_4 : CPW + CD (10%)	10.46	0.418
T_5 : CPWW + CD (10%)	10.68	6.21
T_6 : CPW + CPWW + CD (10%)	12.83	0.510
T_7 : CPW + AC (10%)	12.87	0.515
T_8 : CPWW + AC (10%)	14.03	7.66
T_9 : CPW + CPWW + AC (10%)	16.74	0.669

CPW, Coffee pulp waste; CPWW, coffee processing waste water; CD, cow dung slurry; AC, Anaerobic consortia.

sing wastes was studied in terms of total biogas produced per gram of TS added. In this regard, coffee processing waste water with anaerobic consortium 10% and cowdung slurry as inoculum produced 7.66 and 6.21 L of biogas per gram of TS added and with coffee pulp waste, 0.515 and 0.418 L per gram of TS added respectively. The reactor with both CPW and CPWW inoculated with anaerobic consortium and cowdung slurry recorded 0.669 and 0.418 L of biogas per gram of TS added respectively (Table 5).

Change in microbial dynamics during anaerobic fermentation of coffee processing wastes

The population of total anaerobes, cellulolyzers and acid formers were found to increase up to 30th day of anaerobic digestion and afterwards recorded a gradual decline. Among the treatments, T_9 with CPW, CPWW and anaerobic consortium 10%, registered maximum population of total anaerobes, cellulolyzers and acid formers of about 43 x 10^5, 30 x 10^3 and 24 x 10^2 CFU ml^{-1} respectively followed by T_7 with CPW with anaerobic consortium 10% (28 x 10^5, 23 x 10^3 and 19.0 x 10^2 CFU ml^{-1} respectively) on 30th day of anaerobic fermentation. On the other hand, the population of methanogens recorded a gradual increase and reached maximum at 60th day of anaerobic digestion. In general the maximum population of methanogens was recorded in treatments receiving anaerobic consortium as inoculum followed by cow dung slurry. Similar to other anaerobes, T_9 (CPW, CPWW and anaerobic consortium 10%) registered maximum population of total methanogens (28 x 10^4 CFU ml^{-1} of sample) followed by T_7 with CPW with anaerobic consortium10 per cent (25 x 10^4 CFU ml^{-1} of sample) on 60th day of anaerobic fermentation.

Techno-economic feasibility of anaerobic consortium with cowdung slurry for anaerobic fermentation of coffee processing wastes

The Techno-economic feasibility of anaerobic consortium with cowdung slurry for anaerobic fermentation of coffee processing wastes is shown in Table 6. So, 27.2% of the expenditure on electricity can be saved by using biogas as fuel generated from coffee pulp wastes employing anaerobic consortium.

DISCUSSION

Co-culturing and Methanogenesis

Clostridium sp. TCW 3 and TCW 5 associated with *Methanosarcina* sp. TCWMS 5 under co-culture condition resulted in significant reduction of pectin, tannic acid and cellulose and generated significant amount of biogas. The co-culture of *Clostridium* sp. with *Methanosarcina* sp. accumulates methane with partial utilization of H_2 involved.

The results show that *Clostridium* sp. (TCW 5) possess a pectin methyl esterase that catalyses the hydrolysis of methyl esters linked with the production of methanol and pectic acids. The production of methanol and isopropanol was proportional to the amount of pectin introduced. Similarly, *Clostridium* sp. is able to ferment sugars into isopropanol via acetone-butanol fermentation. Since *Methanosarcina* sp. is a methylotrophic methanogen, it can reduce methanol to methane using the small amount of H_2 produced during the hydrolysis of pectin and cellulose. This shows the existence of interspecies hydrogen transfer and is in accordance with the findings of Ollivier and Garcia (1990). They reported that more than 95% of the methane can be obtained only by associating *C. thermocellum* with *Methanosarcina* sp. In the presence of methylotrophic *Methanosarcina* sp., methanol was reduced to methane without effect on pectin hydrolysis and a small amount of the H_2 produced was also used to reduce methanol. Studies on mehanogenic fermentation have been reported in mesophilic conditions for a bacterial co-culture by Rhode et al. (1981).

The presence of interspecies hydrogen transfer in the system demonstrated a well marked syntrophic association between the two partners which is a good indi-

Table 6. Techno-economic feasibility of anaerobic consortium with cowdung slurry for anaerobic fermentation of coffee processing wastes.

Detail	Anaerobic consortium (10%)	Cow dung slurry (10%)
Total biogas produced per 250 grams of coffee processing wastes	16.74 L (0.017 m^3)	12.83 L (0.013m^3)
Total biogas per Kg of coffee processing wastes	0.068 m^3	0.052 m^3
Total biogas per ton of coffee processing wastes	68 m^3	52 m^3
For coffee processing unit generating 5 tonnes of wastes with an approximate power consumption of 2000 KW hr units		
Biogas from 5 tonnes of coffee processing wastes	340 m^3	260 m^3
1 m^3 biogas = 1.6 KW hr units		
Biogas from 5 tonnes of coffee processing wastes equivalent to	544 KW h units	416 KW h units
Revenue at of Rs 30/KW h unit	Rs.16,320	Rs. 12,480
Cost of electricity (for 2000 KW h units at Rs.30/KW hr unit)	Rs.60,000	Rs. 60,000
Net saving interms of power (%)	27.2	20.8

cator of the promise of this anaerobic consortium. Though the pH of the system dropped down to acidic during the course of fermentation, the methanogenic activity is maintained due to the existence of interspecies hydrogen transfer within the community. There would have exhibited a shift in acetate degradation from direct acetate cleavage towards syntrophic acetate oxidation coupled with hydrogenotrophic methanogens as described earlier (Hao et al., 2013). The syntrophic acetate oxidizing (SAO) bacteria and hydrogenotrophic methanogens (HM) including *Methanomicrobiales* and *Methanobacteirales* were more tolerant to the stress from high acetate concentration and high pH. Resumption from alkali condition to normal pH stimulated the growth of acetate oxidizing syntrophs. These results point to the possibility to regenerate the deteriorated anaerobic digesters by addition of acclimatized inocula rich in acetate-oxidizing syntrophs.

Development of anaerobic consortium and its survival studies

For a successful consortium, the performance as well as the survival of the organisms in a single new platform is very important. Static population of the individual strains upto three weeks showed that significant level of compatability exists among the individual strain which is a mandate for developing any microbial consortium. The results reveal that positive interactions existed among the anaerobic bacteria and the archae. Although there was a dissimilar pattern of reduction in the populations of individual bacterial cultures, which can be attributed to the intrinsic growth characteristics, the comparatively lower counts illustrated the time taken for initial acclimatization (lag phase) and the loss of viability at much later stages.

Efficiency of anaerobic consortium for anaerobic fermentation of coffee processing wastes

During anaerobic fermentation, gradual decline in EC was noticed and maximum reduction was observed in treatments with 10% anaerobic consortium as inoculums. This represents a good operation condition of the system which favours hydrolysis, acidogenesis followed by acetogenesis and methanogenesis. TS reduction of 67% was recorded by the treatment imposed with the developed anerobic consortium rather than cowdung slurry which is conventionally used as an inoculums source. This shows faster metabolic rate of the individual strains in the anaerobic consortium than generalized microbes in the cowdung slurry.

The reduction of biopolymers during anaerobic fermentation of coffee pulp waste might be due to the conversion to simple monomers like glucose and maltose in the first phase of digestion. Pectin and tannic acid content were reduced by 62.27 and 27.94% respectively in the reactor with anaerobic consortium. Boopathy (1988) reported that cellulose, hemicellulose and protein were reduced by 39.43, 58.2 and 43.81% respectively during anaerobic digestion of coffee pulp waste with digester slurry as inoculum. The same trend was observed in the study with cowdung slurry which recorded 51.25, 15.94, 47.44and 45.85% reduction in pectin, tannin, cellulose and hemicellulose respectively. The anaerobic degradation of gallotannins was first reported by Field and Lettinga (1987) who observed the breakdown of tannic acid by a consortium of anaerobic sludge bacteria. When gallotannins were presented at subtoxic concentration, there was a high conversion of tannins into methane. In the present investigation, the per cent reduction of tannic acid was very less (27.94%) when compared to cellulose and hemicellulose. This might be due to the complex structure of tannins which

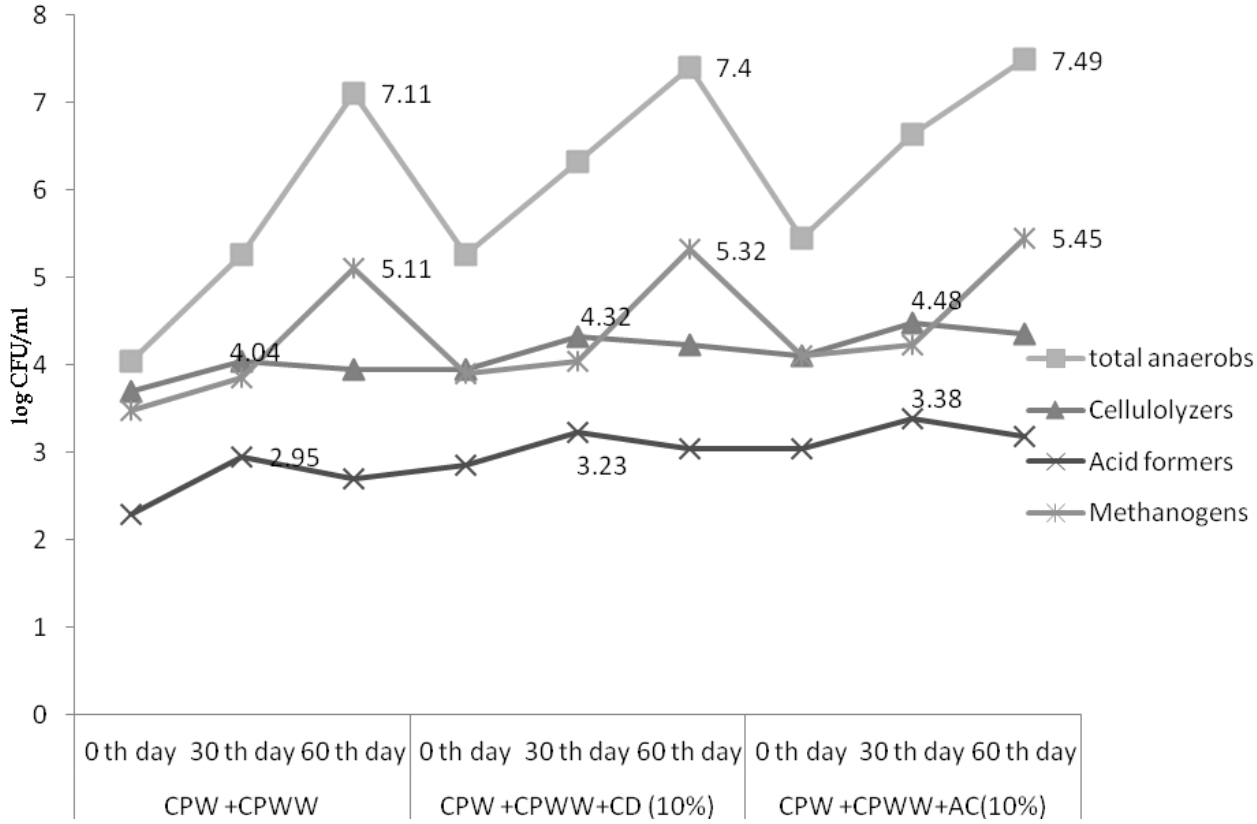

Figure 3. Population dynamics during anaerobic fermentation of the coffee processing wasters.

was not easily hydrolysed. This could be overcome by pre treatment of feed with acid or alkali.

The initial increase of reducing sugars could be attributed due to the hydrolysis of the complex polymers and its subsequent reduction in the later stages is caused by the conversion of reducing sugars to volatile fatty acids and Hydrogen molecules. This confirmed well functioning of the system with succession of microbes.

Methanogenesis and Methane recovery

The average biogas production in reactor with CPW and CPWW with cowdung slurry as inoculums was 4.2 m^3 and methane recovery was 62%. In this case also, TS reduction increased with increase in time, but more stability was attained and no noticeable disturbance occurred in the system. From the results its known that the the biogas production prolonged one week more in reactor with cowdung slurry as inoculum than anaerobic consortium but the cumulative yield is less. The advancement in the reactor with anaerobic consortium is due to earlier build up and acclimatization of the inoculated cells and depletion of nutrients in later stages. From the results, it can be concluded that anaerobic consortium as inoculum performed better than cow dung slurry as inoculum for anaerobic fermentation of coffee processing wastes (Figure 3).

From the microbial dynamics study during anaerobic digestion, it was inferred that in the first phase of anaerobic fermentation of coffee processing wastes, the population of acid formers and cellulolytic bacteria increased. Similar increase population was observed by Boopathy (1988). The methanogenic population was found to be higher after 30th day and maintained till the end of the experiment.

Maximum methanogenic population was observed in coffee waste digester with anaerobic consortium (10%). This might be due to the presence of viable and active methanogenic organisms with the ability to adapt a new environment. This finding confirms that the developed anaerobic consortium is very good inoculum for initiating microbial loads in the anaerobic fermentor.

Techno-feasibility of anaerobic consortium developed in the study

From the study, it was inferred that approximately 27.2% of expenditure on electricity can be saved. Biogas generated during anaerobic fermentation of 5 ton of coffee pulp waste is equivalent to 544 KW h units of

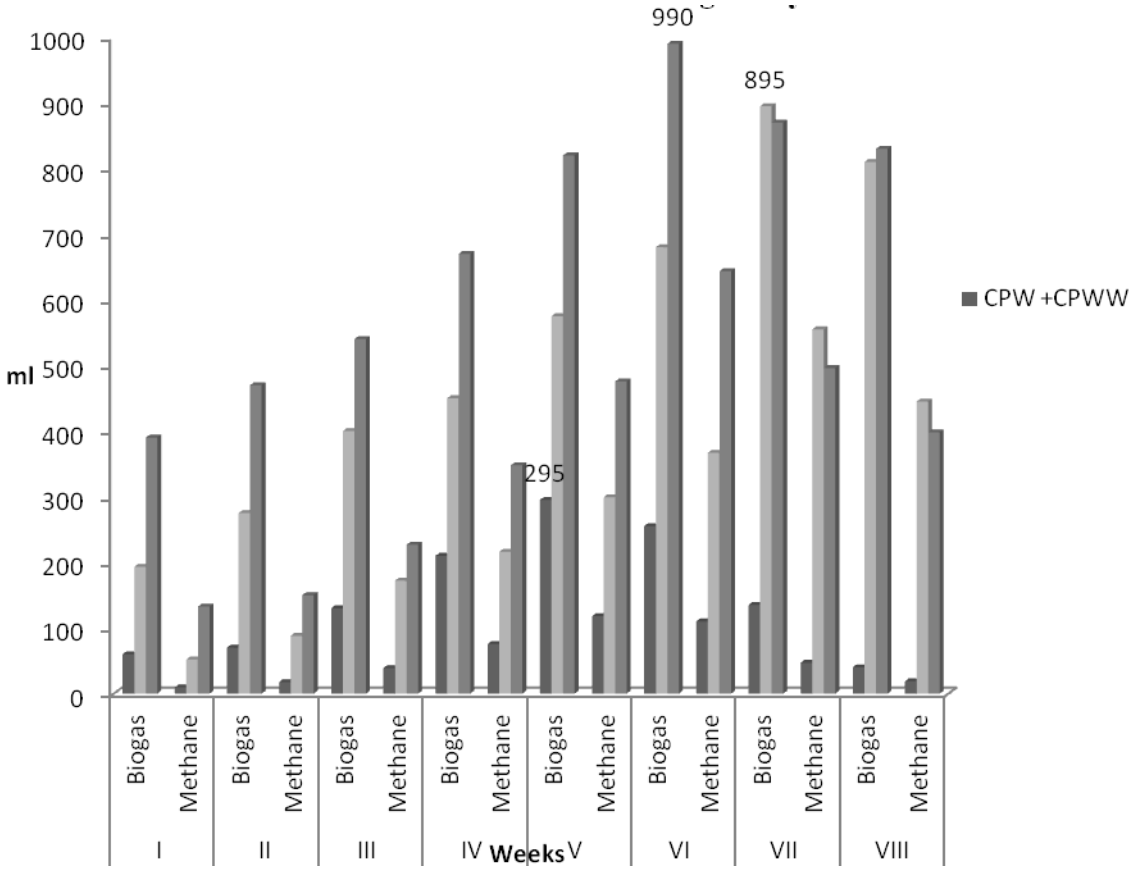

Figure 4. Comparison of biogas and methane production by anaerobic consortia with cowdung slurry.

electricity which can replace 27.2% of total expenditure on electricity. According to previous studies, the potential daily yield of biogas from 2 tonnes of coffee pulp was approximately 131 m^3, equivalent in terms of its fuel value to 100 L of petrol. Boopathy and Mariappan (1984) reported a maximum biogas production of 52 m^3 with coffee waste, cow dung and old slurry in the ratio of 3:1:1.

Conclusion

Among the two inoculum sources, anaerobic consortium and cowdung slurry, the anaerobic consortium developed in the present study showed best results interms of biogas and methane yield with highest consistency of TS removal and pH of the coffee processing wastes. The anaerobic consortium developed could retain methanogenic biomass and other anaerobic bacterial load which could be used for treating coffee wastes without any dilution or neutralization. This has provided a new concept of subjecting very high strength coffee processsing wastes for biomethanation employing anaerobic consortium under high organic loading rate. The post methanation solids obtained can be used as manure and the effluent still needs to be treated to meet the pollution control standards. The bioenergy can be converted interms of electricity saving 27.2% of power consumption with anaerobic consortium.

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