

Evaluation of Biogas Production by Anaerobic Digestion of Duckweed (*Lemna minor*) and Cattle Manure

Alemayehu Negassa¹ Dajene Fikadu²

1.Environmental Sciences Program, Department of Biology, Ambo University, Ethiopia

2.Olankomi Comprehensive Secondary School, West Shoa, Ethiopia

Abstract

This study entailed evaluation of biogas production by anaerobic digestion of duckweed (*Lemna minor*) a plant that can be made easily available as much cheaper feedstock and cattle manure. Total solids, volatile solids, and organic carbon content of duckweed and cattle manure and pH of the slurries were determined using standard procedures. Three sets of plastic bottles were interconnected through connecting tube method. Digestion of duckweed to cattle manure in different percentage ratio (100:0, 75:25, 50:50, 25:75, 0:100) was performed respectively each in triplicates at 38°C temperature for 30 days at Ambo University plant Science Department laboratory. The cumulative biogas production in milliliters from 100% duckweed, 75% duckweed and 25% cattle manure; 50% duckweed and 50% cattle manure; 25% duckweed and 75% cattle manure, and 100% cattle manure was found to be 1015.5, 1040, 1159, 1206, and 862, respectively. Statistical analyses indicated significant differences between means of the physico-chemical parameters determined before and after anaerobic digestion ($P < 0.05$) in all samples. The result revealed the attractive potential of duckweed as a feedstock in biogas production which peaked when the plant was co-digested with cattle manure at ratio of 25% to 75%.

Keywords: Biogas, Renewable Energy, Cow Manure, Duckweed, Rumen Fluid

DOI: 10.7176/JETP/11-2-01

Publication date: March 31st 2021

Introduction

The dependence on fossil fuels as primary energy source has led to global climate change, environmental degradation, and human health problems, and about 80% of the world's energy consumption still originates from combusting fossil fuels (Goldemberg and Johansson, 2004). Besides, the rural population in developing countries including Ethiopia heavily depends on traditional fuels, such as fire wood, animal wastes and agricultural residues with quite significant adverse environmental impacts. Fast rate of population growth with increased energy demand and climate change have emerged as the most crucial issues that made researchers, practitioners and different organizations to search for renewable energy sources (Gokhale *et al.*, 2006). Moreover, rising energy prices and concerns about long term sustainability have once again brought renewable energy sources like biogas to the forefronts.

Biogas is produced through digestion of organic matter by anaerobic bacteria with end products consisting mainly of the combustible gas, methane (CH₄) and a liquid effluent (Rilling, 2005). Biogas can be readily converted to electrical and thermal energy via a co-generator, typically for onsite consumption (Wickham *et al.*, 2016). It is a proven eco-friendly technology that contributes to the reduction of the deforestation rate and helps to save the trees to sequester more carbon from the atmosphere and the local effects of trees being cut down that otherwise cause soil erosion, desertification, loss of soil fertility, and landslides (Mary *et al.*, 2007). Though evaluation of biogas production from different organic materials such as cattle manure and organic kitchen waste (Tamrat Aragaw, 2012), poultry litter (Ebrahim Ali, 2006) and Khat (*Catha edulis*) waste (Tesfaye Negussie, 2007) has been reported so far no research has been conducted on duckweed in Ethiopia. Duckweed is the smallest flowering aquatic plant of immense biogas potential and can easily grow abundantly in nutrient rich waters including waste water with minimum cost and be made available as much cheaper feedstock. This study attempted to evaluate biogas production by anaerobic digestion of duckweed (*Lemna minor*) and Cattle manure.

Materials and Methods

Duckweed and cattle manure were used as substrates and rumen fluid was used as inoculum for biogas production in the experiment. About 15kg of wet duckweed and 5kg of fresh cattle manure were collected from wastewater ponds and dairy farm available at Ambo University main Campus, respectively. The collected substrates were dried with sun light and manually sorted to remove foreign materials and kept in a refrigerator at 4°C until used for the experiment (Wendland *et al.*, 200). The only biomass preprocessing that was required in this study was grinding into smaller uniform sized particles for samples of substrates.

Fresh rumen was taken from stomach of cow sacrificed for its meat and filtered through 0.5 mm sieve diameter to separate solid content from slurry. The rumen was added to the digesters and kept in anaerobic condition following Aurora (1983). The study was conducted based on five samples of the substrates (Table 1) which were properly mixed before adding to the digesters.

Design of the experiment

Duckweed (DW) and cattle manure (CM) were prepared as samples separately and also mixed in three different combinations. The experiment was done in triplicates for each sample. Thus, 15 digesters were charged once during the experimental period of 30 days. Since the focus of this study was mainly about biogas production, the experiment was conducted in a batch mode and all digesters were placed in an oven dry that was adjusted at 38°C.

Determination of the physico-chemical properties of the samples

Total solids (TS), volatile solids (VS) and organic content of samples of substrates and pH of the slurries were determined following the Standard Methods given in APHA(1999).

Total solids

Clean crucibles were stored at 105 °C for 1hour and then the mass of the empty crucibles were determined and recorded. Next, the mass of crucibles were measured with 6g of samples of substrates. Then, the crucibles that contain samples were placed in oven at 105°C for 24 hours. This was mainly aimed to evaporate all the water from the samples to determine total solids (TS). Then, the crucibles containing dried samples of substrates were removed and cooled in desiccators. The mass of total solids was determined by subtracting mass of crucible from mass of crucible containing samples

The percentage TS was calculated using the following formula (APHA, 1999).

$$\% \text{ TS} = \frac{\text{mDS}}{\text{mFS}} \times 100$$

Where: %TS = percentage of total solids, mDS = mass of dried samples of substrates (g) and mFS = mass of fresh samples of substrates (g),

The percentage of TS removal was calculated using as:

$$\text{TS removal}(\%) = \frac{\text{TSi} - \text{TSf}}{\text{TSi}} \times 100$$

Where: TSi = initial total solid (TS before AD) and TSf = final total solid (TS after AD)

Volatile solids

After total solids (TS) were determined, the oven dried samples of substrates that exist in crucibles were ignited at 550°C in a furnace for 3 hours. Next, crucibles that contain ash were cooled in desiccators and their masses were measured. The mass of volatile solids was determined by subtracting mass of crucible that contain ashes from that contain dried samples. The following formula was employed to calculate the percentage of volatile solids content of the samples of substrates (APHA, 1999).

$$\% \text{ VS} = \frac{\text{mDS} - \text{m(ignated sample)}}{\text{mDS}} \times 100$$

Where: %VS = percentage of volatile solids, mDS = mass of dried samples of substrates (g), m (ash) = mass of ignited samples of substrates (g)

The percentage of VS removal was calculated using the equation below.

$$\text{VS removal}(\%) = \frac{\text{VSi} - \text{VSf}}{\text{VSi}} \times 100$$

Where: VSi = initial volatile solids (VS before AD) and VSf = final volatile solids (VS after AD)

pH

The pH before and after AD was determined using digital pH meter (HANNA HI 8314). An electrode was inserted into samples of substrates that were diluted using distilled water before inoculation of rumen fluid for measure pH before AD and the pH measurement after AD was done using pH electrode inserted into the fermentation slurry of substrates samples that were digested for about 30 days (Elijah, *et al.* 2009).

Organic carbon

Carbon content of the feedstock was obtained from volatile solids data using an empirical equation developed by Badger *et al.* (1979) as:

$$\% \text{ Carbon} = \frac{\% \text{ VS}}{1.8}$$

Where, VS = Volatile solids

Determination of total fluid added to digesters

The anaerobic digestion of substrates samples were conducted in batch mode in 0.6 liter plastic bottles as digesters labeled for all substrate samples (Table 2) and 100.62g of rumen fluid was added into all digesters of samples of substrates. To get 8% of total solids in the fermentation slurry, the total liquid (distilled water and rumen fluid)

that was added to the samples of digesters was independently determined following Tchobanoglous *et al.* (1993) as:

$$mTF = \frac{mTS - 8\% mFs}{8\%}$$

Where: mTS = mass of total solids (g), mFs = mass of fresh samples of substrates (g) and mTF = mass of total fluid (g)

To achieve the recommended (8% m/m) total solids content in the fermentation slurries, the mass of distilled water that has to be added was determined as below:

$$mdw = mTF - 100.62g$$

Where: mdw = mass of distilled water (g) and mTF = mass of total fluid (g)

Biogas reactor

Sets of five plastic bottles each with 0.6 liter capacity were used as digesters. This was a modification of a compact system digester that digests small volumes of duckweed and cattle manure to produce biogas. The second sets of five plastic bottles with 0.6 liter capacity were used to contain acidified solution, and the third sets of other five plastic bottles were used as conical flasks each with 0.5 liter capacity. All the three set containers were interconnected with a plastic tube in batch mode (Elijah *et al.*, 2009). The acidified brine solution was prepared by adding sodium chloride to distilled water until a supersaturated solution was formed to prevent the dissolution of biogas in the water following Tamrat Aragaw (2012). The supersaturated solution was formed by dissolving 40g of sodium chloride into 100ml of water at 20°C. Then, the five substrates samples were loaded into their digesters and each digester was connected to each of the second plastic bottle filled with acidified brine solution by means of a connecting tube.

Then, three drops of sulphuric acid were added using a dropper to acidify the brine solution. The biogas produced in the digesters by the fermentations of samples substrates passed through the connected tube to the second sets of plastic bottles containing acidified solution. The pressure of the biogas produced in the second bottles caused a displacement of acidified solution through a connected tube into the third sets of plastic bottles. Thus, the biogas produced by fermentation of the slurry was driven from the first sets of bottles to the second sets of bottles so as to displace a volume of the brine solution into the third sets of bottles which is equivalent to the volume of biogas produced. This displaced solution was measured by 250 ml of measuring cylinder (Itodo *et al.*, 1992) in every 2 days in a month which represents the amount of biogas produced

As biogas production commenced in the fermentation chamber, it was delivered to the second chamber which contained the acidified brine solution. Since the biogas is insoluble in the solution, a pressure build-up provided the driving force for displacement of the solution. The displaced solution was measured to represent the amount of biogas produced.

Since the experiment was done in triplicates, all samples of substrates triplicated in independent digesters and the main contents of the slurry in each digester were determined (Table 2).

Laboratory Analysis

All apparatus were properly cleaned and allowed to dry in the laboratory. Analyses of samples were done by taking some contents of the samples (about 6 grams). Fresh duckweed had high water content and hence only 560g of the dried form was obtained from 15kg of fresh duckweed collected. For the anaerobic digestion, 30g of substrate(s) was/were used. Sample size of substrates was determined based on the dry mass of duckweed and the volume of digesters used for AD. Total solids and volatile solids were determined based on standard procedures (APHA, 1999). The carbon content of the feedstock was obtained from volatile solids data using an empirical equation as reported by Badger *et al.* (1979). The pH of the fermentation samples of substrates in each digester was measured and recorded using a pH meter. Paired simple t-test was run to compare physio-chemical parameters before and after AD.

Results and Discussions

The total solid content of all samples before AD ranged from 87.33% to 91.45% from 6 grams of the samples while that after AD ranged from 78.66% to 84.66%(Table 3). The maximum TS before AD was measured in duckweed whereas the minimum was documented in cattle manure. High content of volatile fatty acids (VFAs) in the substrates can produce misleading results in TS and VS since they might volatilize from the substrate when they are first heated and thus, give total solids and volatile solids values that are too low. This in turn can produce incorrect estimates of biogas production, which depend on volatile solids (Annaschnure, 2010). However, it did not occur in the present research study.

After AD, values of TS significantly decreased in all samples of substrates compared with that before AD (Table 3). However, the extent of decrement was more pronounced in mixed samples of substrates than these parateones. Removal of TS suggests conversion to biogas and high removal was observed in 25% DW and 75%

CM indicating the highest biogas production from the sample.

Statistical analyses indicated significant differences ($P < 0.05$) in TS before and after AD in all samples. The results revealed that total solids had positive impacts on biogas production by anaerobic digestion of duckweed and cattle manure.

The volatile solids before AD ranged from 78.51% to 80.49% and from 74.75% to 75.85% after AD. High reduction of VS was measured in 25% DW and 75% CM mix substrates as compared to the rest samples of substrates (Table 4). Removal of VS also suggests its conversion to biogas.

Total solids and volatile solids reduction are a good parameter for evaluating the efficiency of anaerobic digestion (Abubaker and Ismail, 2012) and it is a good indicator of biogas production (Anonymous, 1981). Similar results were reported by Joung *et al.* (2008) who studied methane production potential of anaerobic co-digestion from swine manure and food waste.

The volatile solids determined for duckweed and cattle manure substrates were 78.31% and 76.53%, respectively. This is in accordance with Fulford (1988) who reported the volatile solids in animal and human wastes in the range from 77% to 90%. The ash content of duckweed was found to be much lower than cattle dung which indicated lower amount of non-biodegradable fractions of carbon and hence higher digestibility. This is in accordance with Yadav *et al.* (2017) who reported much greater ash content of cattle manure as compared to that of duckweed. Mean volatile solids before AD are statistically significantly different from the mean volatile solid after AD in all samples of substrates ($P < 0.05$).

pH and Organic Carbon

The pH values of slurries after AD increased as compared to that before AD (Fig. 3). This indicates that the rumen fluid used for this study had a good buffering capacity as reported earlier by Girma *et al.* (2004), Forster-Carneiro *et al.* (2008), Montusiewicz *et al.* (2008), and Uzodinma and Ofoefule (2008). The pH values of all slurries samples before AD ranged from 6.03 to 7.33 while that after AD ranged from 7.63 to 8.60 (Fig. 1). These results were in accordance with previous reports by Thy *et al.* (2003) and Yadvika *et al.* (2004). The pH value of 100% CM was 7.33 which is optimum for biogas production, whereas that of 100% DW was 6.03 which is less optimal and in agreement with Thy *et al.* (2003) and Yadvika *et al.* (2004). Slurries of mixed samples of substrates resulted in the rise of pH compared to that of DW, but a decrease in pH from CM slurry. The results indicated that co-digestion is a good way of adjusting the pH value to the optimum for duckweed, and in line with Hills and Roberts (1981). Before AD, the pH was found to increase significantly with increasing of CM proportion in the mix suggesting that CM helps to maintain the pH to meet the optimum required. Increased pH values after AD might be due to the production of ammonia resulting from high organic matter available in duckweed than cattle manure which is supported with Gray *et al.* (1971). The statistical results indicated that the mean pH before AD is statistically significantly different from the one after AD in all samples ($P < 0.05$).

In all samples reduction of organic carbon was observed after AD (Fig. 2) which might be because organic carbon can be assimilated, transformed, and decomposed by bacteria in anaerobic digestion process. This observation was in accordance with Anonymous (1981) and Gerardi (2003) which state that organic carbon can be removed in anaerobic digesters either by being converted to cellular materials for growth and reproduction of bacteria or biogas production. This is also in accordance with Devlin *et al.* (2011) who attributed the decrease in organic carbon to the degradation process during anaerobic digestion. The percent degradation of organic carbon for 25% DW and 75% CM was the highest compared with the rest samples of substrates, suggesting that mixing can enhance degradation and biogas production. Similar result has also been reported by Teame (2014) from experiment on co-digestion of cow dung and cactus peel. The statistical results indicated significant difference ($P < 0.05$) between mean organic carbon (OC) values before and after AD in all samples.

Evaluation of biogas production

Within initial six days high biogas production was observed from digestion of the digester containing only cattle dung as the feedstock (Fig. 3). This may be due to the presence of higher amount of easily degradable organic carbon fractions in the cattle dung which is in line with Seppala *et al.* (2013) who reported presence of labile carbon sources in cattle dung that could be easily hydrolyzed by acidogenic and acetogenic bacteria. Provenzano *et al.* (2011) attributed the point to the fact that cattle dung contains more simple degradable monomers fermentative bacteria degrade these molecules to volatile fatty acids and to ammonia; methane formation occurs in which the acids are converted to biogas.

The production of biogas in the initial days shows that microorganisms did not need time to acclimatize to the substrate due to active inoculum and in agreement with Abdien (2003). Also the fact that gas production occurred at the beginning of the experiment suggests the existence of microbes in the added rumen fluid inoculum to act on readily degradable materials of the substrates was concluded by Kamthunzi (2008) and (Teame, 2014). The biogas production from duckweed and cattle manure mixtures exceeded that from samples of each substrate due to the balanced (nutrient to microorganism) composition and stable pH which was attained from the

inoculation with rumen fluid and mixing ratios used.

The amount of biogas produced from 100% DW exceeded the amount produced from 100% CM and peaked in 25% DW and 75% CM (Fig.4). This might be due to more availability of biodegradable material in DW than CM to serve as a source of energy for microbes in full process of digestion and in agreement with Hobson *et al.* (1981). Macias-Corral *et al.* (2008) also states that biogas production is a function of the feedstock's organic content and its biodegradability. The least production resulted in 100% CM probably might be partly due to the partial fermentation that usually takes place in the intestinal tract of the animal as reported by Deublein and Steinhäuser (2008). Duckweed being a lignocellulosic biomass contains lignin and cellulose which needed more time to break down into more labile carbon moieties and thus gets converted into precursor for methanogenesis as reported by Facchin *et al.* (2013). The higher production from the mixtures could be due to a proper nutrient balance, increased buffering capacity, and decreased effect of toxic compounds resulting from mixing of the substrates (Fulford, 1988; Macias-Corral *et al.*, 2008; Li *et al.*, 2009).

As the proportion of DW in the mix ratio increased from 25 % to 75 %, the cumulative biogas yield decreased which is in agreement with Yadav *et al.* (2017). This observation was also in accordance with the results of an experiment done by Callaghan *et al.* (1999) using Water hyacinth, poultry manure and cow dung, where higher cumulative biogas production was produced in the system with the lower concentration of water hyacinth. This might be due to the high concentration of total nitrogen (ammonia) resulting from anaerobic breakdown of proteins to inhibit anaerobic digestion (Angelidaki and Ahring, 1999). Thus, co-digestion of CM and DW was more productive with DW proportion not exceeding 25%.

The present experiment indicated that duckweed has potential for biogas production. In addition to this, the percentage of volatile solid from total solid content of the duckweed substrate was 78.31% whereas that from Cow dung was 76.53% which indicated that a large fraction of duckweed was biodegradable. Based on the total production volume of biogas during a hydraulic retention time of 30 days, the optimum mixing for digestion of duckweed to cattle manure has been found to be at 25%:75% ratio. Biogas produced from duckweed was 15.17% greater than that produced from cattle manure.

Conclusions

The biogas produced from DW exceeded the amount produced from CM which indicated the potential of duckweed as important feedstock in biogas production. The study further revealed that anaerobic co-digestion of 25% DW and 75% CM might constitute optimum levels for better biogas production.

Acknowledgments

We would like to thank the Holeta Agricultural Research Center and Ambo University Plant Science Department for allowing us to use laboratory spaces and necessary facilities. We also thank Melkamu Fikadu for his continuous technical supports.

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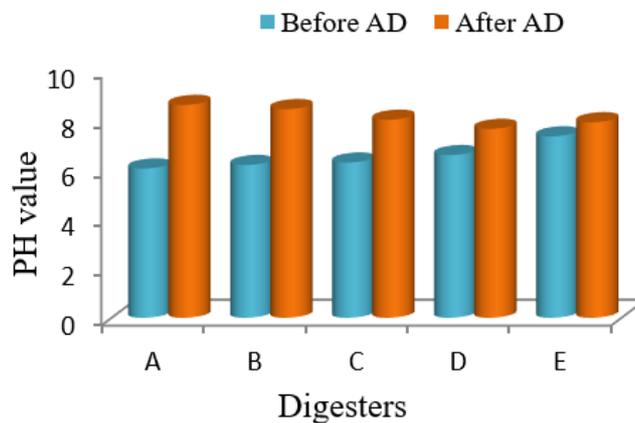


Fig.1 pH of slurries in digesters

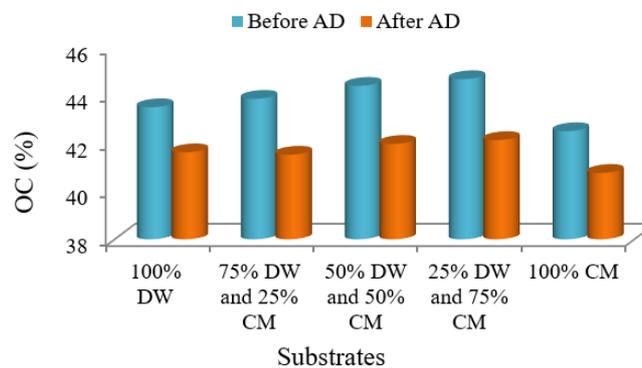


Fig.2 Organic content (OC) of samples of substrates

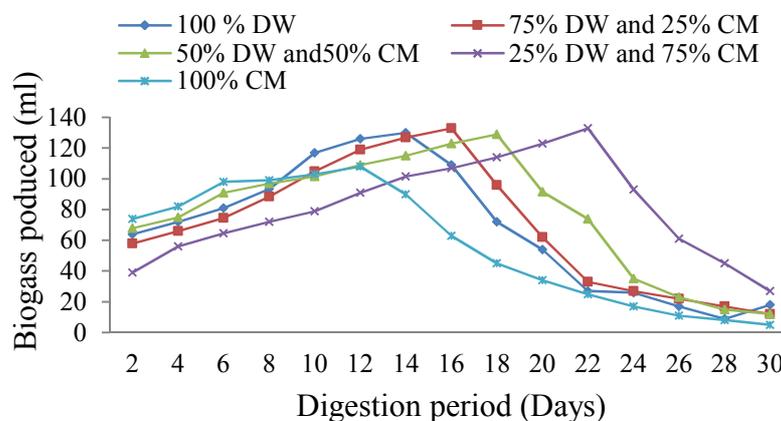


Fig. 3 Biogas produced from anaerobic digestion of the different samples

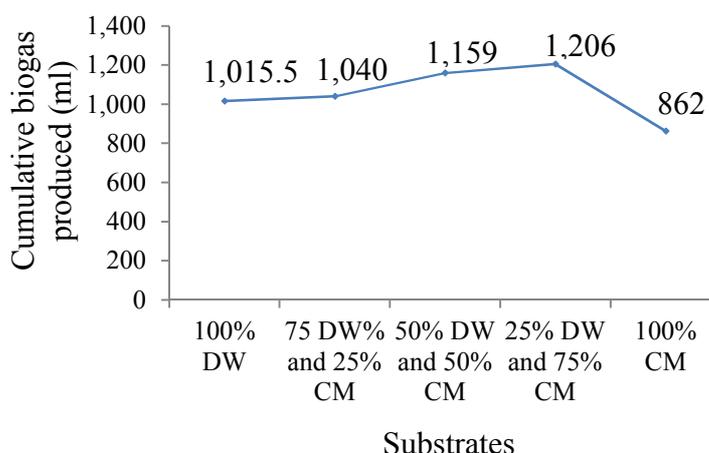


Fig.4 Cumulative biogas produced from digestion of duckweed and cattle manure

Table 1. Samples of substrates used for the experiment

Samples of substrates	Corresponding digesters
100% Duckweed	A
75% Duckweed and 25% Cattle manure	B
50% Duckweed and 50% Cattle manure	C
25% Duckweed and 75% Cattle manure	D
100% Cattle manure	E

Digesters A and E served as controlling groups while B, C and D as experimental groups.

Table 2. Contents of initial slurries in each digester

Digesters	Mix ratio (%)	Fresh TS before AD (g)	Distilled water added into digesters (g)	Rumen fluid added into digesters (g)	Total mass of fluid (g)	
A	100 0	30 0	27.45	212.50	100.62	313.12
B	75 25	22.5 7.5	27.15	208.75	100.62	309.37
C	50 50	15 15	26.65	202.50	100.62	303.12
D	25 75	7.5 22.5	26.40	199.38	100.62	300.00
E	0 100	0 30	26.20	196.88	100.62	297.50

Table 3. Total solids removal from 6g of samples of substrates

Sample	Before AD		After AD		Removal	
	(g)	(%)	(g)	(%)	(g)	(%)
100% DW	5.49	91.45	5.08	84.66	0.41	7.42
75% DW and 25%	5.43	90.55	4.99	83.16	0.44	8.16
50% DW and 50% CM	5.33	88.83	4.83	80.50	0.50	9.38
25% DW and 75% CM	5.28	87.95	4.72	78.66	0.56	10.56
100% CM	5.24	87.33	4.85	80.83	0.39	7.44

Table 4. Volatile solids from mass dried samples of substrates

Samples of Substrates	before AD		after AD		Removal	
	(g)	(%)	(g)	(%)	(g)	(%)
100% DW	4.31	78.51	3.81	75.00	0.50	4.47
75% DW and 25%	4.29	79.00	3.73	74.75	0.92	5.38
50% DW and 50% CM	4.26	79.92	3.65	75.57	0.61	5.44
25% DW and 75% CM	4.25	80.49	3.58	75.85	0.67	5.76
100% CM	4.01	76.53	3.56	73.40	0.45	4.09