

## **Optimization of Anaerobic digestion of cattle manure Effect of its association with the aquatic weed *Pistia* (*Pistia stratiotes*)**

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### **Abstract**

This study investigates the improvement of performance of anaerobic fermentation of cattle manure mixed with the water lettuce *Pistia stratiotes*, a macrophyte plant growing in the effluent of an anaerobic digester.

The experiments used a series of continuous fermentors, using mixtures of cattle manure and water lettuce in the following proportions: 12.5 %, 16.6 %, 25 % and 50 % of *Pistia stratiotes*. The best biogas yields were achieved with a proportion of 50 % of water lettuce in the mixture giving a biogas yield of 0.62 m<sup>3</sup>/ (m<sup>3</sup>.d) with a methane content of 76.8 % over a 15 days hydraulic retention time, at a constant temperature of 35 °C. The kinetic study based on batch fermentation shows that the process is well represented by the Monod Model. These performances are better than those obtained in anaerobic digestion of cattle manure used alone.

**Key words:** Anaerobic digestion, cattle manure, aquatic weed, biogas, *pistia stratiotes*

### **Résumé**

#### **Optimisation des performances de la digestion anaérobie des déchets de bovins. Effet de leur association avec la plante macrophyte (*Pistia stratiotes*)**

La présente étude envisage de tester les performances de la digestion anaérobie des déchets de bovins associés à la laitue d'eau ou *Pistia stratiotes*, plante macrophyte cultivée sur effluent de digesteur méthanique. Les essais ont été réalisés dans une série de digesteurs en batch et en continu pour des substrats constitués par un mélange de déchets de bovins et de laitue d'eau aux différentes proportions P<sub>1</sub>= 12,5 %, P<sub>2</sub>= 16,6 %, P<sub>3</sub>= 25 % et P<sub>4</sub>= 50 % de laitue d'eau.

Les meilleurs résultats ont été obtenus pour la proportion P<sub>4</sub>=50 % de laitue d'eau, donnant une production en biogaz de 0,62 m<sup>3</sup>/ (m<sup>3</sup>.j) contenant 76,8 % de méthane pour un temps de séjour de 15 jours, à une température d'expérimentation de 35 °C. L'étude cinétique du système montre que le processus est bien représenté par le modèle de Monod. Les performances obtenues sont

meilleures que celles qui sont réalisées au cours de la fermentation anaérobie des déchets de bovins utilisés seuls.

**Mots-clés:** digestion anaérobie, déchets de bovins, plantes macrophytes, pistia stratiotes, biogaz

## ملخص : تحسين إنتاجية البيوغاز من روث الأبقار بمزجه مع خس الماء (*Pistia stratiote*)

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تهدف هذه الدراسة إلى تحسين إنتاجية البيوغاز من روث الأبقار بمزجه مع خس الماء (*Pistia stratiote*) المزروع على مصب الهاضم الميثاني.

أجريت التجارب في مجموعة من الهواضم المستمرة بمادة خام مكونة من خليط روث الأبقار و خس الماء بنسب مختلفة 12.5، 16.6، 25 و 50 بالمائة من خس الماء. أحسن النتائج المحصل عليها بنسبة 50 بالمائة من خس الماء، أعطت إنتاجا من البيوغاز يعادل 0.62م<sup>3</sup>/3م<sup>3</sup>/يوم محتويا على 76.8 بالمائة من الميثان في مدة 15 يوما في حرارة تعادل 35 درجة مئوية. بينت دراسة حركية إنتاج البيوغاز أنها أكثر تطابقا مع نموذج مورود حيث كانت المرودية أحسن من تلك التي حصلنا عليها من خلال التخمر اللاهوائي لروث الأبقار وحده.

**كلمات مفتاحية :** الهضم اللاهوائي، روث الأبقار، البيوغاز، التنقية، خس الماء (*Pistia stratiote*)، الترشيح

## Introduction

Rural populations (12.7 millions; Direction de la statistique 1995) in Morocco use either forest wood and straw or fossil fuels as energy sources for their cooking and other household needs. With the increasing costs of fossil fuels and the necessity to protect natural forests from further degradation, it becomes crucial to develop alternative energy sources to satisfy totally or partially the needs of rural households. An alternative way is the production of biogas using anaerobic digestion (fermentation) of cattle manure. This process has been widely

used throughout the world (Hashimoto and *al.* 1981; Dubourguier *et al.* 1985); however, several authors pointed out the limited performance of this system due to the influence of physico-chemical parameters of the fermentary process. Recently, several researchers investigated ways to improve the performance of the fermentary process through the addition of ground plant material, especially that of the water lettuce *Pistia stratiotes*, an aquatic plant that can be grown on effluent ponds.

The objective of this study is to test the performance of biogas production in an integrated system incorporating the water lettuce. This study is the first of its type in Morocco.

## Materials and methods

The experiments were carried out on the Energetic Platform at the experimental farm of the National school of agriculture of Meknes. This platform is composed of several reactors with effluents recuperated in ponds supporting macrophyte plants: *Pistia stratiotes*, *Eichhornia crassipes* and *Lemna gibba*. This process associates, at the same time, gas production, plant biomass production and eruption of waste waters. (fig.1)

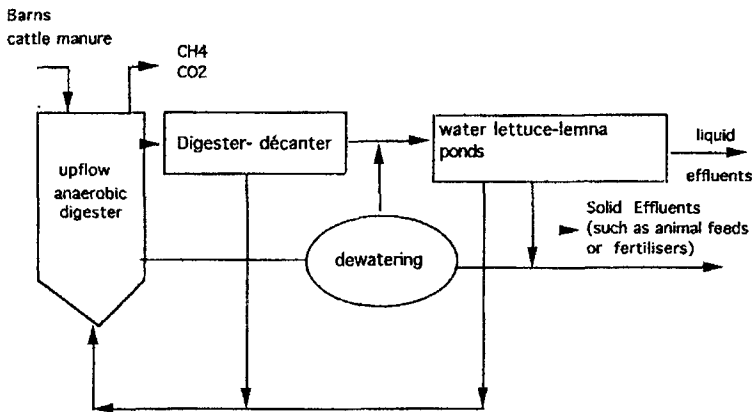


Figure 1. Schematic diagram of intergrated macrophytes wastewater treatment and biogasification system

### The substrate

Fresh cattle manure was obtained from the barns, carefully isolated from straw and diluted to the desired concentration.

Water lettuce has been chosen for its large productivity (Reddy and *al.* 1983). The introduction of this plant in the system has many advantages: 1) the purification of the effluent through active absorption of organic matters and minerals (Nelson *et al.* 1981; Ignjatovic and Marjanovic 1986; Reddy and Debusk 1987; 2) its use as animal feed (Sauze, 1981; Wolverson and McDonald, 1981; Charbonnel, 1989); 3) the enhancement of biogas production when ground and added to the

fermenting substrate and 4) its aesthetic aspect and its low investment (Sauze 1981)

This macrophyte was provided from the "Exotic gardens" in Rabat. It has been acclimated and maintained in ponds alimented by the fermentors effluent. Harvesting occurred at maximum growth of the plant which was weight, grounded and associated to cattle manure for experimentation.

### **The digestors**

Two types of digesters were employed in these studies: separate completely mixed bioreactors had been conducted at continuous mode in order to test the effect of temperature and the addition of ground water lettuce biomass. These reactors consisted of 70 liters polyethylene cylindric containers, where the temperature was kept at 35 °C and 55 °C  $\pm$  0,5 °C by circulating water. The fermentors were operated at 15 days hydraulic retention time.

In the same way, the kinetics of water lettuce-cattle manure biomass biomethanation was studied at batch mode in 500 ml glass serum flask.

### **Procedures**

The quantities used to start the experiments were: 26.66 kg of cattle manure diluted at 90 g liter<sup>-1</sup> and 7.3 kg of water lettuce diluted at 90 g liter<sup>-1</sup>. As soon as biogas is produced, the fermentors were fed each day by 4 liters of the mixture substrate diluted at 40 g liter<sup>-1</sup>. The different proportions tested were: P<sub>1</sub> = 12.5 %; P<sub>2</sub> = 16.6 %; P<sub>3</sub> = 25 % and P<sub>4</sub> = 50 % of water lettuce.

At batch mode fermentation the experiments were conducted at 35 °C and 55 °C. The concentrations used were of 40 g l<sup>-1</sup>, 50 g l<sup>-1</sup> and 80 g l<sup>-1</sup> of dry weight basis(DW) at 35 °C, and of 40gl<sup>-1</sup> and 80gl<sup>-1</sup> of DM substrate at 55 °C.

An experiment was done in the same conditions with a substrate of cattle manure alone.

### **Chemical analysis**

Total solids (TS) were determined by drying the samples at 105 °C to constant weight; Volatile Solids (VS) were determined by combustion of the dried sample at 620 °C . Chemical oxygen demand (Cod) and mineral content were determined according to the standards methods for water analysis (Rodier 1984; Apha 1985). The total nitrogen (Kj-N) concentration was determined using Kjeldahl method. Volatile fatty acids (Vfa) and gas analyses were carried out using gas chromatography. pH measurements were done with conventional glass electrodes.

## **Results and discussion**

### **Fermentation substrate characteristics**

Table 1 shows the relevant chemical characteristics of the substrate used during the course of the experiment. It is composed of cattle manure alone and mixtures of cattle manure and ground water lettuce, It appears clearly that mixtures have higher mineral contents, with concentrations increasing with the proportion of the water lettuce biomass in the mixture. These minerals are essentially composed of phosphorous, calcium, sodium and potassium. Furthermore, the

amount of nitrogen was also higher in mixture than in pure cattle manure. Nitrogen concentration was also increasing with the proportion of water lettuce in the mixture. Globally, we may say that there is an enrichment of the substrate in minerals and in proteins which is provided by the water lettuce.

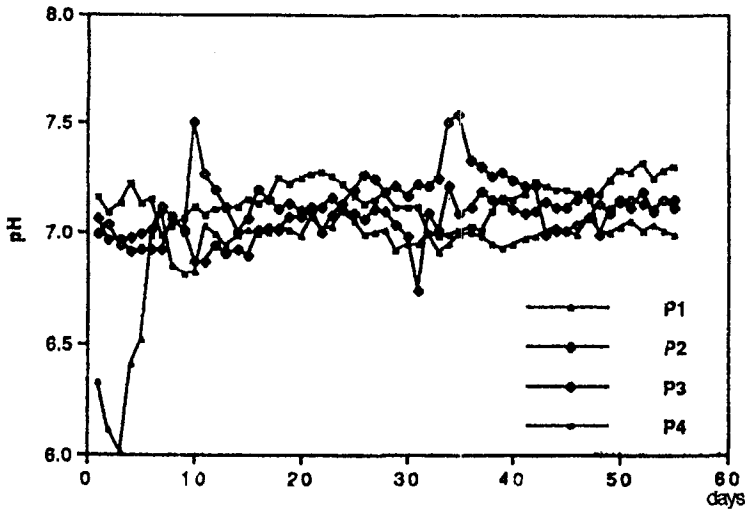
**Table 1.** Characteristics of cattle manure and cattle manure-water lettuce mixtures used as substrates in the reactors. (dry weight basis)

Parameters	Cattle manure pure	Mixture substrates with proposition of w. lettuce			
		P1 12.5 %	P2 16.6 %	P3 25 %	P4 50 %
Total Solids g liter <sup>-1</sup>	16.38	36.38	36.70	36.15	38.6
V .Solids (%)	85.50	83.21	81.61	80.47	9.19
Org. carbon (%)	45.35	43.12	40.20	39.00	40.14
Cellulose (%)	32.50	26.50	24.78	24.15	29.12
Nitrogen kj (%)	1.54	4,01	2.13	2.22	2.48
C/N	29.44	21.45	18.87	19.31	16.13
Phosph. (%)	0.50	0.45	0.64	0.881	1.16
Sodium (%)	1.00	1.57	1.85	2.10	3.60
Potassium (%)	1.05	3.42	3.35	3.45	4.85
Calcium (%)	5.25	6.325	6.35	6.35	7.05
C.O.D.mg 0 <sup>2</sup> /l	9227	7208	8995	10151	11950
VFA mg acet.ac./l	1327	1378	1255	1239	1047
pH	6.73	6.99	7.03	7.03	7.09

### Fermentation process

#### *Buffer property of the digestor*

Figure 2 shows pH variation along the whole period of fermentation for the different substrates. It can be noted from this figure that pH is maintained in a neutral zone, except for proportion P<sub>1</sub>, which shows at the beginning an acid pH reaching neutrality after 5-6 days. This pH variation is clearly related to the Volatile fatty acids (Vfa) concentration in the mixture. This demonstrates the buffering power of the system, that can be explained by the existence of a high proportion of nitrogen of which the part transformed to ammonia serves to counterbalance the sudden increase of Vfa. The system may also be buffered by cations, particularly sodium and calcium which form salts with organic acids, preventing the latter from inhibiting the process by reducing the pH.



**Figure 2.** pH variation during fermentation of substrate composed of 4 different mixtures of cattle manure and *Pistia stratiotes* biomass at 35 °C and Hydraulic retention time (HRT) of 15

#### *The Volatile Fatty Acids*

Figure 3 describes the evolution of VFA for the 4 different mixture substrates. It appears that the P<sub>1</sub> substrate shows instability in the concentration of Vfa fluctuating between 200 mg.l<sup>-1</sup> and 800 mg.l<sup>-1</sup>, reaching even 1200 mg.l<sup>-1</sup> of acetic acid. For the proportions P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub>, we observe a very high stability. Globally, it can be clearly seen that VFA concentration decreases as the proportion of the water lettuce in the mixture increases.

Globally, the Vfa concentration remains low in the four mixtures and far from the toxicity limit which is 2.000 mg.l<sup>-1</sup> acetic.ac. (Sinechal and *al.* 1979). These data show the good performance of the fermentation process in the mixture substrate. However, what is worth observing is the decrease of Vfa as the plant proportion increases. This can be explained by the existence of a bacterial flora adapted to the substrate that balances the pH and enhances good degradation of Vfa leading to their depletion from the system. This is well illustrated in figure 4 showing the evolution of biogas production for the different mixture substrates. Effectively, we notice that biogas yield evolves in the reverse direction of that of Vfa; the best production of biogas corresponds to the lower concentration of Vfa and hence to the proportion P<sub>4</sub>=50 % of the plant. This confirms the fact that, for this proportion, the methanogenic activity is more important and quicker than the acidogenic activity, resulting in a maximum use of the Vfa, avoiding in this way their accumulation in the bath.

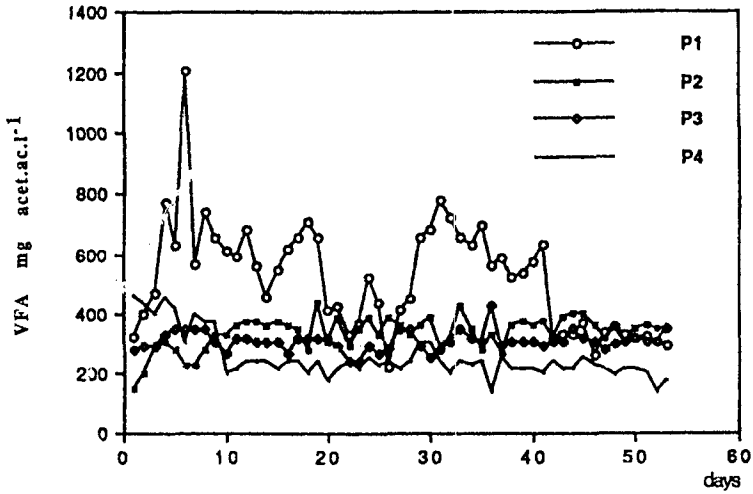


Figure 3. Volatile fatty Acids (Vfa) evolution during fermentation of substrate composed of 4 different mixtures of cattle manure and *Pistia stratiotes* at 35 °C and Hrt = 15d

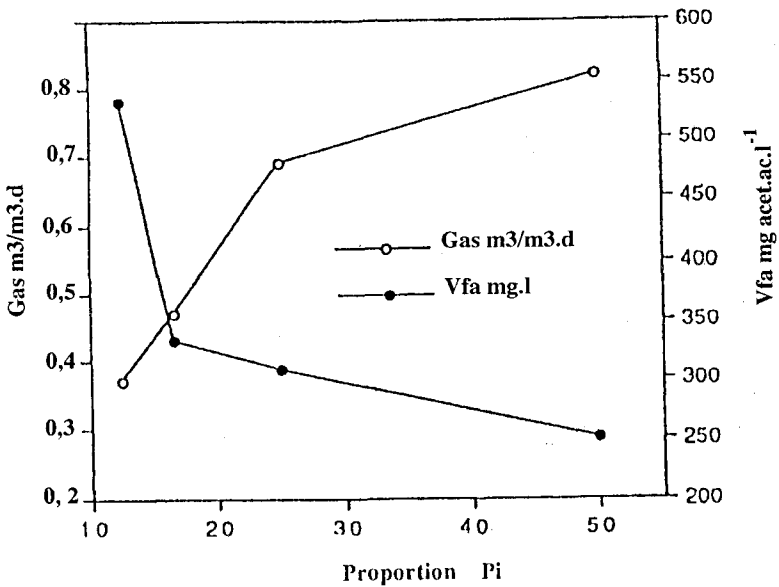
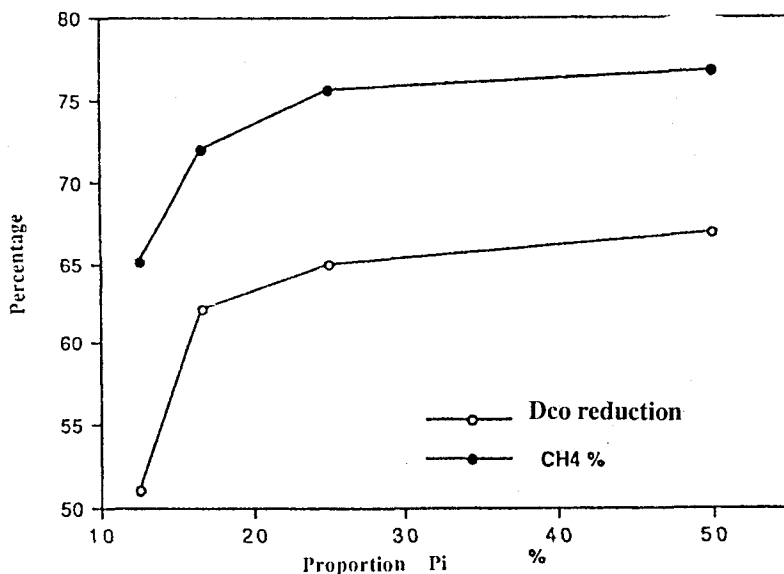


Figure 4. Biogas production and Vfa evolution for the 4 mixtures of cattle manure and *Pistia stratiotes* at 35 °C and Hrt = 15d

### Methane production and efficiency of eparation of the effluent

Figure 5 illustrates the evolution of methane content in biogas and the reduction rates of the Chemical oxygen demand (Cod) for P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub>. It can be seen that the proportion P<sub>1</sub> goes together with an increase in methane content in biogas and with a reduction of Cod. Consequently, we can say that the best production of biogas occurred for the P<sub>4</sub> proportion which leads to 0.62 m<sup>3</sup>/m<sup>3</sup>.d with a methane content of 76.84 % and the larger reduction rate of Cod of 67 %.



**Figure 5.** Evolution of Dco réduction biogas methane content for the 4 mixtures of cattle manure and *Pistia stratiotes* at 35 °C and HRT = 15d

The high methane content seems to come from the fact that the substrate contains important quantities of proteins parallel to that of the water lettuce proportion in the mixture. In fact, according to Hawkes (1980) proteins release during the anaerobic degradation a gas with a high methane content of 84 % This value which is higher than the maximum obtained in our studies can be justified by the fact that our substrate contains in addition to proteins important quantities of sugars .

Our results confirm that the increase of water lettuce proportion in the mixture enhances methanogenic activity leading to better yield of methane with the best yield being achieved by the 50 % proportion of water lettuce in the mixture. Taking into account the results obtained by the fermentation of cattle manure alone (table 2), our data demonstrate an improvement of the fermentary process by the addition of water lettuce ..



**Table 2.** Biogas yields from fermentation of pure cattle manure and cattle manure -water lettuce mixture substrates

Parameters	Cattle manure Pure	Mixture substrates with proportion of w.lettuce			
		P1 12.5 %	P2 16.6 %	P3 25 %	P4 50 %
Biogaz m <sup>3</sup> /m <sup>3</sup> j	0.42	0.37	0.43	0.60	0.82
CH <sub>4</sub> m <sup>3</sup> /kg O.M. input	0.15	0.06	0.10	0.19	0.37
CH <sub>4</sub> m <sup>3</sup> /kg C.O.D. input	0.52	0.23	0.32	0.55	0,60

### Conclusion

The association of water lettuce to cattle manure substrate produced interesting results. It increased markedly the activity of methanogenic bacteria. Also, this mixture produced a synergistic effect because methane production in this experiment was higher than that of the substrate composed of cattle manure alone. Hence, it seems that the high nitrogen content and the presence of much minerals had a beneficial effect on the methanogenic activity. We confirm, therefore the results obtained by Nipaney and *al.* (1987) and Abbasi and *al.* (1991) in their experimentation on *pistia stratiotes* and Madawmar *et al.* (1990) in their experimentation of *Eichhornia crassipes*.

### Kinetics study

The objective of this part of the study was to establish a data base for employing a kinetic model that will predict methane yield and production rate as a function of substrate concentration. These experiments provide a uniform basis for comparison of our results with those of fermentation of cattle manure alone.

The kinetic models used were developed by Monod (1942), Andrews (1975) and Chen and Hashimoto (1980) for growth kinetic applied to bacterial growth of cattle manure in anaerobic digesters. The best fit was obtained with the Monod model.

#### Monod kinetic model

Because of the complexity of the bacterial process in anaerobic digestion, we considered the reactions as a one step system :

Complex organic matter + microorganism + water  $\longrightarrow$  microorganism + methane + carbon dioxide

The specific rate at which the substrate disappears can be given as the sum of three terms: one for the growth of the cells, one for product development, and another for maintenance of cells, each of them being multiplied by a stoichiometric factor Y. Thus:  $-1/X \cdot (dS/dt) = Y_{(s/x)} \mu_x + Y_{(s/p)} \mu_p + m$

where  $\mu_x$  is the specific growth rate of cells and  $\mu_p$  is the specific rate of methane formation, S is the effluent biodegradable substrate concentration (g/ l) and t is the time.

Monod gave a general expression for  $\mu$  with respect to the limited substrate :

Specific growth of the cells model ----->  $\mu_x = \mu_{x\max} \cdot S / (K_s + S)$

Specific methane production model ----->  $\mu_p = \mu_{p\max} \cdot S / (K_p + S)$

where :

$\mu_{x\max}$  is the maximum specific growth rate of cells ( $d^{-1}$ )

$\mu_{p\max}$  is the maximum specific product rate (methane) ( $d^{-1}$ )

$K_s$  is the saturation constant of substrate (g/l)

$K_p$  is the saturation constant of methane production (g/l)

The actual values of the rates of methane production, cell growth, and substrate disappearance were determined by fitting our experimental data adjusted with the predicted model. The parameters of the models were determined by non linear regression and the model selected is the one possessing the smallest residual sum of square. (Table 3 and 4)

*Specific growth rate model:*

At 35 °C (table 3), it is observed that the maximum specific growth rate value is not dependant on the substrate concentration, confirming in that way the results obtained in the bibliography (Chen and Hashimoto 1980; Andrews and *al.* 1982). Furthermore, it is lower for cattle manure substrate, showing thus, a best activity of cells in the mixture substrate. These values are not significantly different from the results obtained by Chen *and al.* (1981):  $0,27d^{-1}$  on glucose and pepton and Henze and Harremoes (1983):  $0,3d^{-1}$  for methanogens activity. This confirms that, in our case, the methanogenic activity is more important than acidogenic activity.

At 55 °C, the maximum specific growth rate increases with temperature (table 4). As demonstrated by Andrews *and al.* ( 1982) this relation is expressed by the equation:  $\mu_{x\max} = 0.013T - 0.129$

**Table 3.** Kinetic constants determined during batch fermentation of cattle manure substrate and mixing of cattle manure-water lettuce substrate at 35 °C

Substrate	Cattle manure			Cattle- manure	water- lettuce	mixed
Concentration % DW	4.00	5.00	8.00	4.00	5.00	8.00
$\mu_{x\max} d^{-1}$	0,26	0,27	0,27	0,31	0,33	0,33
$K_s$ g/l	40,27	54,12	64,42	22,63	33,56	51,77
$\mu_{p\max} d^{-1}$	0,52	0,50	0,51	0,38	1,37	1,91
$K_p$ g/l	40,56	55,45	63,28	18,60	35,2	51,70

**Table 4.** Kinetic constant determined during batch fermentation of cattle manure substrate and mixing of cattle manure-water lettuce substrate at 55 °C

Substrate	Cattle manure		Cattle manure-water lettuce mixed	
Concentration % DW	4,00	8,00	4,00	8,00
$\mu_{x\max}$ d <sup>-1</sup>	0,62	0,64	0,54	0,61
Ks g/l	40,44	56,21	19,08	43,06
$\mu_{p\max}$ d <sup>-1</sup>	0,79	0 80	1,15	3,84
Kp g/l	40,58	58,37	25,00	23,00

*Methane production rate model:*

Maximum specific rate of methane formation increases with temperature and with biomass concentration. It is more important for the mixture, including thus, the positive influence of the water lettuce in the mixture. This effect is confirmed by Ks and Kp values which are higher for cattle manure used alone.

*Substrate utilization model:* Table 5 presents the constant values of the substrate utilization model for the two temperatures 35 °C and 55 °C. It appears that in the two cases, the growth cells yield,  $Y_{x/s}$ , is insignificant in comparison with that of methane production,  $Y_{x/p}$  on the other hand, it is noted that when the temperature increases  $Y_{x/s}$  decreases although  $Y_{x/p}$  increases, confirming thus data experimented by Madawmar and *al.* (1991); therefore it seems that we have not any advantage to work in the thermophilic zone and fermentation in mesophilic temperature would be a good alternative, for a project of design plant.

**Table 5.** Substrate utilization rate constant in anaerobic fermentation in batch for water lettuce alone and cattle manure-water lettuce mixture at 35 °C and 55 °C. (concentration: 8 % DW)

Substrate	Cattle manure- w.lettuce mixt	
	35	55
Temperature		
$Y_{xs}$	0,026	0,016
$Y_{CH_4}$	0,223	0,408
m	0,117	0,045
$R^2$	0,92	0,78

**Conclusion**

The addition of water lettuce biomass to cattle manure improves the fermentary process over that realized with cattle manure alone. The best results characterized by a biogas production of 0,82 m<sup>3</sup>/m<sup>3</sup>.d with a methane content of 76.8 % are obtained by the proportion  $P_4=50$  % of water lettuce which presents a

composition allowing a good activity of methanogenous bacteria, and a good equilibrium between the different flora, as indicated by the absence of the environmental stress. The process is characterized by high stability, a normal running and accordingly the achievement of results of epuration associated to a satisfactory production of biogas and a high methane content in biogas .

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