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TWO-PHASE LIQUID STATE AND SOLID STATE FERMENTATION OF WATER HYACINTH

(*Fermentasi cair dua fase dan fermentasi padat eceng gondok*)

By/*Oleh*

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Ringkasan

Tulisan ini menyajikan percobaan dua teknologi fermentasi biogas dari eceng gondok (Eichhornia crassipes). *Fermentasi cair dengan dua phase menghasilkan 1 volume biogas per volume reaktor per hari dengan volume beban 4 kg bobot kering per meter kubik reaktor per hari dan pemakaian waktu 12,5 hari. Sedangkan fermentasi padat menghasilkan lebih banyak biogas, yaitu 3-4 volume biogas per volume reaktor per hari dengan volume beban 15 kg bobot kering per meter kubik reaktor per hari dan pemakaian waktu 22 hari. Kedua teknologi ini memberikan produksi biogas yang lebih tinggi daripada sistem konvensional seperti batch, Gobar dan semi-kontinu. Terutama fermentasi padat memberi harapan walaupun memerlukan praperlakuan eceng gondok secara intensif. Selain itu suhu merupakan faktor penting. Pada suhu 35°C (mesophilik); produksi gas lebih tinggi dibandingkan pada suhu ruang (22-28°C). Fermentasi pada suhu mesophilik memungkinkan juga penambahan volume beban menjadi lebih tinggi.*

I. INTRODUCTION

Since the beginning of the seventies, biogas generation or methane fermentation received a lot of attention because of two reasons. Firstly, the end product, biogas, a mixture of methane and carbon dioxide, is a combustible gas with similar properties as natural gas and likewise represents a source of energy. Secondly, through fermentation organic matter is anaerobically (i.e. in the absence of oxygen) degraded to a non-hazardous gas. Hence, the process can be beneficial for environmental technology in cases where organic waste has to be dealt with.

Two-phase liquid state fermentation (LSF) is a relatively new technology, which is mainly applied for the digestion of wastewaters. It is based on the knowledge that the conversion of organic polymers to biogas is a serial multistep process in which each step is executed by a different group of micro-organisms. A schematic view of these different conversions is presented in Figure 1. By separating the digestion over two phases, a liquefying phase consisting of hydrolysis and acidogenesis, and a methanogenic phase consisting of acetogenesis and methanogenesis, the different microbial groups can each be given optimal conditions with regard to physiology, nutritional requirements and growth rate (Ghosh and Klass, 1978).

As only liquid components are going through the methanogenic reactor, it becomes possible to maintain the methanogenic bacteria, which grow very slowly,

Polymers (carbohydrates, proteins, lipids)
↓ Hydrolysis
Monomers (sugar, amino acids, glycerol, etc.)
↓ Acidogenesis
Alcohols, volatile fatty acids
↓ Acetogenesis
Acetate, H_2/CO_2
↓ Methanogenesis
CH_4, CO_2

Figure 1. Schematic survey of the main processes involved in anaerobic digestion (De Zeeuw, 1984)

Gambar 1. Skema proses utama pencernaan anaerobik (De Zeeuw, 1984)

indefinitely in the reactor. This is achieved by their attachment to a carrier matrix.

Solid state or dry fermentation (SSF) is a new technique used for the conversion of solid substrates. It consists of a digestion at a total solids concentration (TS) of 20% or higher. In nature this occurs spontaneously at landfill sites. On a rather simple basis the productivity of a reactor can be increased substantially. Indeed, if a completely mixed reactor at a TS of 5% can produce 1 V/Vr.d of biogas, a reactor operating at 30% TS should produce 6 V/Vr.d if there is no significant inhibition at higher solids concentrations. For the digestion of municipal solid waste, TS of 30% (Carantino, 1983) and 30% to 40% (De Baere & Verstraete, 1984) were applied. Gas productivities of respectivily 3 and 4 to 5 V/Vr.d were obtained at mesophilic temperatures.

As a substrate for biogas generation, water hyacinth, *Eichhornia crassipes*, has received a lot of attention. This tropical water plant combines a very high growth rate (up to 150 t DM/ha.yr) with a broad ecological spectrum. In most parts of the tropical and subtropical world it has evolved as a persistent pest. Its conversion to biogas offers both the advantages of energy production and pest control. Moreover the residue of the reactor can be used as a fertiliser/soil conditioner.

In the framework of project ATA-251, a lot of research has been carried out at the Forest Products Research and Development Centre (FPRDC) on LSF and SSF of water hyacinth. This article gives an overview of the results of the most relevant experiments.

II. MATERIALS AND METHODS

Water hyacinth was obtained from several ponds belonging to the FPRDC in Bogor. The total surface of the ponds was about 500 m^2. Immediately after harvesting, the plants were chopped with a hammermill driven by a motor of 5 HP. For SSF they had to undergo an additional pretreatment in order to increase the TS concentration. This was achieved by sun drying and/or pressing with a hydraulic cylinder press.

LSF experiments were done both on lab-scale and on small pilot-scale. The lab-scale reactors had a total volume of 2 l and existed of one liquefying reactor (LR) and one methanogenic reactor (MR), each having a volume of 1 l (Figure 2). Liquid is pumped from the LR to the MR and flows back through gravity. Solid particles are maintained in the LR by means of a metal screen. The MR is filled with PUR-foam which acts as a carrier matrix for the bacteria. On regular intervals, depending of the required experimental parameters, the LR was fed with a precise amount of water hyacinth. The pilot-scale LSF unit consists of 4 vessels with a volume of 500 l each. One reactor is filled with PUR-foam and acts as the MR while the 3 others are LR's. A schematic view is given in Figure 3 while a top view with the recirculation pattern is given in Figure 4. The principles are the same as for lab-scale LSF. Feeding was done at regular intervals by emptying a complete LR and filling it up with water hyacinth. In each LR also a manual stirrer is provided for mixing.

For SSF 2 sizes of reactors were used. Both types exist of a closed vessel without any moving parts, connected to a gas measuring system. A first type are 2 liter PVC reactors of which the top and the bottom are closed by a rubber stopper. Another type consist of 20 l metal vessels. One vessel can only be opened at the top, while the other cylindrical vessel can be opened at the top and the bottom by means of a rubber stopper. A schematic view of all types of SSF reactors is given in Figure 5.

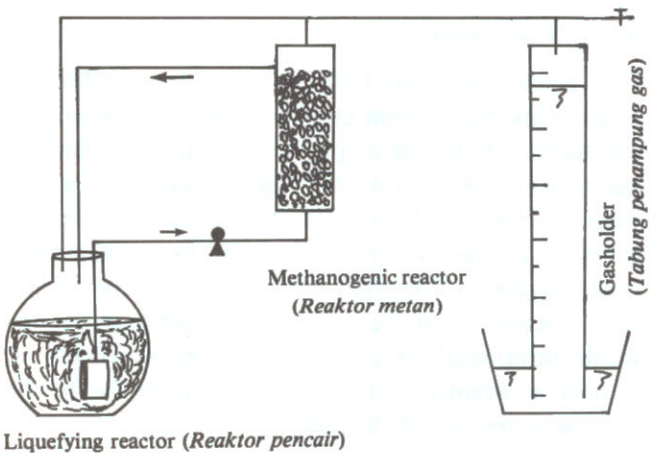
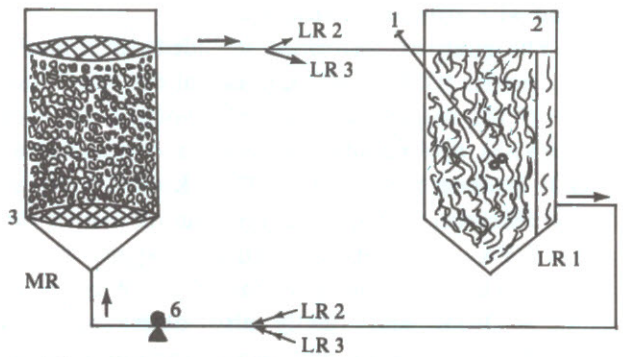


Figure 2. Lab-scale digestor for LSF
Gambar 2. Digester fermentasi basah skala laboratorium



1. Mixer (*Batang pengaduk*)
2. Screen (*Kawat pemisah*)
3. Cylindrical cage with PUR (*Silinder dengan PUR*)
4. MR (*Reaktor metan*)
5. LR (*Reaktor cairan*)
6. Pump (*Pompa*)

Figure 3. Pilot scale liquefying reactor and methanogenic reactor
Gambar 3. Reaktor cairan dan reaktor metan skala pilot

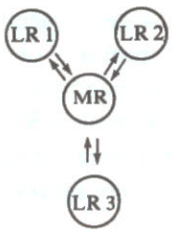


Figure 4. Top view of LSF pilot unit with recirculation pattern
Gambar 4. Pandangan atas unit reaktor fermentasi cair dengan sistem sirkulasi

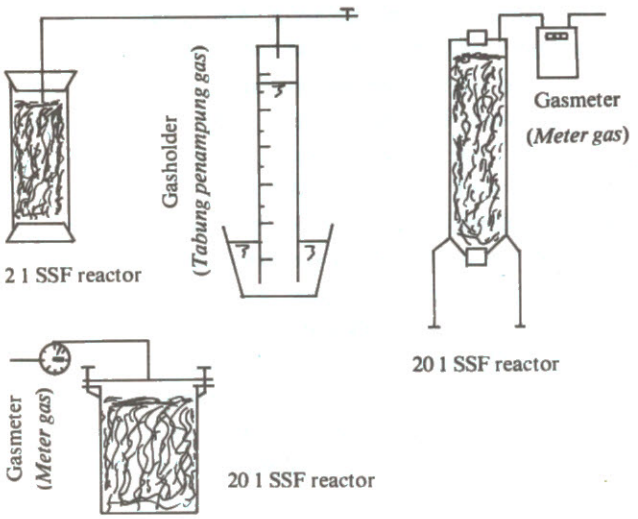


Figure 5. Schematic view of 2 l and 20 l SSF reactors
Gambar 5. Skema reaktor fermentasi padat kapasitas 2 l dan 20 l

In some of the experiments the digestors were heated. For the 2 l LSF and SSF reactors this was done by submersion in a warm water bath. For the 20 l SSF and 2000 l LSF units this was achieved by winding heating wire around the reactors.

The analyses performed included determination of dry matter (DM, at 105°C), ash (at 600°C), volatile matter (VM), suspended solids (SS), volatile suspended solids (VSS), determination of chemical oxygen demand (COD) (Standard Methods, 1971), Kjeldahl nitrogen (Kj-N) (Bremner, 1960), determination of volatile fatty acids (VFA) and volatile chemical oxygen demand (VCOD) (Deutsche Einheitsverfahren zur Wasseruntersuchung, 1975) and finally also determination of hemicellulose, cellulose and lignin fraction (Chesson, 1977).

III. RESULTS AND DISCUSSION

A. Water hyacinth

The composition of the water hyacinth used in the experiments was continuously analysed. The results are summarised in Table 1. A distinction is made according to the origin of the water hyacinth. The number of analyses reflects the importance of each place of origin. Most substrate was obtained from Cikoneng. Less data for DM are available because in SSF the water hyacinth was previously dried before analysis and utilisation. Therefore those data are not representative for the fresh feedstock.

The influence of the depth of the pond on the ash content is obvious. This is due to mud attached to the roots of the plant. Also the higher DM content for the plants of Cikoneng can be explained by the high mud contamination.

The COD and Kj-N are for each pond of the same order. The COD is expressed in g/gVM because the oxygen demand, a parameter for the organic content, is determined by VM only. The COD expressed per gDM, which will be more useful to describe the input, is more variable as it also depends of the ash content.

Table 1. Summary of the analyses on water hyacinth
Tabel 1. Rangkuman analisis eceng gondok

Origin (*Asal*)	DM % (*Bobot kering bahan*)	Ash % (*Kadar abu*)	COD (*g/gVM*)	Kj-N (*mg/g DM*)
Cikoneng	7.6 (1.0)*	36.1 (6.0)	1.22 (0.31)	15.1 (4.3)
(d = 19 cm)	(35)°	(69)	(59)	(57)
Ciampea	6.7 (1.0)	24.4 (2.8)	1.23 (0.18)	18.8 (2.1)
(d = 52 cm)	(6)	(9)	(8)	(8)
FPRDC	6.5 (2.0)	16.4 (3.0)	1.10 (0.22)	17.2 (1.9)
(d = 85 cm)	(6)	(13)	(10)	(5)

d = depth (*kedalaman*); * = standard deviation (*simpangan baku*); ° = number of analyses (*jumlah analisis*)

On the average water hyacinth was composed of 10.7% hemicellulose, 26.5% cellulose and 6.0% lignin, all on dry weight basis. The root system accounts for 30-40% of the total biomass. It was observed that this percentage tends to be lower the healthier the plant. Therefore it seems to be appropriate to utilise the complete plant for methane fermentation instead of the leaves only, as it is sometimes suggested in the literature. Moreover, as the selective harvesting of leaves only is technically rather complicated and time consuming.

B. LSF experiments

Several lab-scale LSF experiments were done in order to investigate some conditions such as operational parameters, influence of particle size and influence of temperature. Also two other much occurring water-weeds were included in the research for comparison. An overview of the most relevant experiments with their results is given in Table 2. It should be noted that on this type of experiments the results being averages over the whole duration of the experiments, must be considered to have a variance of 15%. This is mainly due to the varying properties of the feedstock.

Run 1 till 3 indicate that the optimal loading rate for such configuration is around 2.2 gDM/lr.d. In run 4 the water hyacinth used was cut into pieces of 20-30 mm

Table 2. Overview of the results of lab-scale LSF experiments
Tabel 2. Rangkuman hasil penelitian fermentasi cair skala laboratorium

Run (*Ragam perlakuan*)	1	2	3	4	5	6	7	8
Feedstock (*Bahan masukan*)	Ec	Ec	Ec	Ec*	Ec	Ec	Sm	Nn
T	amb	amb	amb	amb	meso	meso	amb	amb
Input DM %	4.1	4.2	5.0	3.2	3.0	7.6	6.1	8.2
R.T. (d)	23.0	19.0	16.6	19.0	16.6	19.0	25.5	16.0
Bv (gDM/lr.d)	1.8	2.2	3.0	1.7	1.8	4.0	2.4	5.1
P (V/Vr.d)	0.4	0.5	0.5	0.3	0.8	1.1	0.4	1.5
E (%)	43	47	32	30	83	64	23	56

with Ec = Eichhornia crassipes chopped, Ec* = Eichhornia crassipes cut.
Sm = Salvinia molesta, Nn = Nelumbo nucifera
T = temperature (*temperatur*)
amb = ambient (*temperatur ruang*)
meso = mesophilic (*suhu mesophilic*)
R.T. = retention time (*retensi waktu*)
Bv = volumetric loading rate (*volume beban*)
P = productivity (*produksi*)
E = efficiency (*efisiensi*), calculated on basis of the COD of the input and the biogas production (methane content of 62.5%) and a theoretical production of 350 ml CH_4/gCOD if 100% converted.

in stead of being chopped with the hammermill as in all other experiments. Chopping reduced the particle size till 1-4 mm. The larger particle size proved to be less suited for biomethanation (compare run 4 versus run 1). When the reactors are kept at a constant mesophilic temperature (35°C) as in run 5 and 6, the performance is markedly improved. Both productivity and efficiency are higher, while also the loading rate can be increased. *Salvinia molesta* (tropical duckweed) shows to be a less suited substrate for biogas generation. On the contrary, *Nelumbo nucifera* (pink lotus) ferments much better than water hyacinth. The reason for this could be the structure of *Nelumbo nucifera*. The plant is rooting in the bottom of the lake and no part is coming out of the water. Hence, the floating, sponge-like structure typical for water hyacinth and *Salvinia molesta*, which might contain difficult degradable carbohydrates, is not present.

An overview of the pilot-scale LSF experiments is presented in Table 3. In all experiments water hyacinth was used as the single feedstock. The active volume of the 3 LR's was each time 400 l. The active volume of the MR was decreased from 500 l in run 1 to 300 l in run 2 and 150 l in all the other runs. This decrease proved to have no influence on the efficiency of the conversion and gave an easy opportunity to increase loading rate and productivity. The optimal active volume of the MR is about 1/8 of the total LR active volume. The optimal loading rate can be situated at around 3.75 kg DM/m^3r.d (runs 1 till 4).

Table 3. Overview of the results of pilot-scale LSF experiments
Tabel 3. Rangkuman hasil penelitian fermentasi cair skala pilot

Run (*Ragam perlakuan*)	1	2	3	4	5	6
T (C)	24.9	25.3	26.9	26.5	34.1	29.4
Input DM %	4.3	3.9	4.7	4.8	4.7	5.3
V (l)	1700	1500	1350	1350	1350	1350
R.T. (d)	30.5	18.7	13.5	12.6	13.5	13.5
V MR/V LR	2/5	1/4	1/8	1/8	1/8	1/8
Bv (gDM/lr.d)	1.4	2.1	3.5	3.8	3.5	3.9
P (V/Vr.d)	0.4	0.6	0.8	0.8	0.8	0.7
E (%)	59	59	50	46	48	46

with V = volume (*volume*)

In run 5 and 6 an attempt was made to work under constant mesophilic temperature conditions. Although the average temperature was higher, it could not be kept constant because of electricity failures and the overload security system of the heating device. The results reveal a low performance. On the average 40% of the biogas was produced in the MR, resulting in a P of 3 V/Vr.d. About 60% came out of the LR which likewise produced at a rate of 0.6 V/Vr.d. The pH was always around 7. The level of soluble COD and VFA remained constantly low (about 1 g/l and 0.4 gHAc/l respectively). This indicates that the process was never disturbed or out of balance and that the rate-limiting step is the hydrolysis of the carbohydrates. A thorough mixing was needed to break flotation in the LR's. This was achieved by withdrawing liquid from the bottom of the reactor and afterwards reintroducing it at the top.

C. *SSF experiments*

In order to use water hyacinth as a single substrate for SSF, it has to be pretreated to adjust the total solids content (TS). Freshly chopped water hyacinth has a TS of about 7.5%. For most experiments this had to be increased up to 30-35%. Several methods were tried out. In a first method, the chopped water hyacinth was primarly pressed at 15 kg/cm^2 resulting in a TS of about 15%, after which it was dried in the sun till the desired TS was reached. If after drying the TS happened to be too high, it was readjusted by adding tap water. This showed to be a rather laborious method which was also hampered by the limited hours of sunshine in the rainy season and the high relative humidity. A minimum of 5

Table 4. Overview of the results of SSF experiments
Tabel 4. Rangkuman hasil penelitian fermentasi padat

Run (*Ragam perlakuan*)	1	2	3	4	5	6	7	8	9	10	11
T (C)	22-28	22-28	22-28	23-33	29-35	32-39	29	32	35	35	35
V (1)	2	15	2	15	2	15	2	2	2	15	2
Input DM (%)	23.1	26.5	32.7	28.0	22.0	24.7	34.2	35.3	33.6	31.9	31.9
R.T. (d)	50	44	25	33	23	26	39	22	21	20	20
Bv (gDM/lr.d)	4.6	6.0	10.4	8.5	9.3	9.5	8.7	16.3	15.2	15.9	15.9
VFA (gHAc/l)	0.6	0.7	0.6	2.3	0.1	0.5	0.3	0.3	0.1	0.2	0.3
P (V/Vr.d)	0.8	0.8	1.0	0.8	2.0	1.7	1.4	2.5	3.7	2.7	3.3
E (%)	43	32	21	22	47	41	38	40	58	42	51

hours intense sunshine are required. In another method, pressures of 30 kg/cm^2 were applied, bringing the TS immediately to 25%. A short post-drying was enough to reach the desired TS. Such a practice proved to be much more efficient. Other variations, such as drying or pressing of whole plants in stead of the chopped material, didn't result in further improvements.

An overview of the most relevant experiments with their results is presented in Table 4. A distinction has to be made between the first 6 runs in which the temperature was variable and the last 5 runs where the temperature was kept constant. The first experiments date from before adequate heating systems could be used. Efficiency was calculated in the same way as for LSF. On Table 4 it can be clearly seen that the temperature of the reactor is of the utmost importance. In runs 1 till 3 the reactors were operated under ambient conditions with temperature fluctuations up to 6 C/d. The P attains a maximum of only 1 V/Vr.d. In run 4 the reactor, protected from rain, was heated by sun radiation. Though during part of the day the temperature was 33°C, the variation was high, giving a low performance illustrated by the high VFA level which indicates an imbalanced fermentation. In run 5 a diurnal cycle at a high average temperature was simulated. P rose to 2 V/Vr.d. In run 6 the average temperature was 35°C, but with irregular variations. The performance was low. In run 7, 8, 9 and 11 the temperature was kept fairly constant. The closer to the optimum of 35°C, the higher the biogas production. A maximum of 3.7 V/Vr.d was achieved. In run 10 the average temperature was 35°C, but due to the security system on the heating device (see also LSF experiments) sometimes it varied. A comparison with run 11, under similar operational parameters, reveals a decrease in P of about 20%. Optimal operational parameters for 35°C are a retention time of about 20 days and a loading rate of 16 gDM/lr.d or 10-11 gVM/lr.d. The resulting productivity is about 3.7 V/Vr.d at an efficiency of conversion between 50 and 60%. The pH never dropped below 7.5 and except for 1 unstable experiment (run 4) the VFA level always remained low. This indicates a balanced fermentation in which hydrolysis is the rate-limiting step.

D. *Discussion*

The most important results of the research on LSF and SSF from water hyacinth are the following. Water hyacinth should be digested as a whole. Separating the leaves to avoid mud contamination wastes too much biomass and energy.

Other waterweeds such as *Nelumbo nucifera* and *Salvinia molesta* can also be readily digested. Nevertheless, their low productivity and standing crop (respectively 4 tDM/ha and 3.5 tDM/ha) in comparison with water hyacinth (10 tDM/ha) makes them unsuitable as single feedstock in an integrated and controlled concept of biomethanation from aquatic biomass.

Particle size reduction of the substrate enhances its digestibility. For both LSF and SSF the temperature of the reactor is from the utmost importance. The closer to the optimum of 35°C and the more constant, the higher the performance. Productivity is 2 to 3 times as high in comparison with ambient temperature conditions.

In all experiments the pH was always neutral and also the level of VFA remained low. This implies there was no acidification, which means that the rate-limiting step of the complete process is the hydrolysis. Consequently, the major advantage of two-phase LSF, being a separate build-up of methanogenic microbial mass, diminishes. Sun drying proved to be insufficient in the pre-drying of water hyacinth for SSF. A mechanical dewatering is advisable.

Conclusive data on LSF and SSF from water hyacinth, at a constant mesophilic temperature, are presented in Table 5 together with data from literature on other fermentation technologies with water hyacinth as sole substrate.

Table 5. Performance comparison of different types of digestion with water hyacinth as substrate

Tabel 5. Perbandingan beberapa tipe digester dengan eceng gondok sebagai bahan baku

Type of digestion (*Tipe digester*)	T (°C)	n	Bv (gDM/lr.d)	P (V/Vr.d)
Batch	ambient	2	1.59 (0.00)*	0.13 (0.05)
Gobar	ambient	4	0.29 (0.26)	0.16 (0.16)
Semi-continuous	35	14	2.44 (1.08)	0.47 (0.22)
Semi-continuous	55	3	4.57 (0.98)	0.87 (0.15)
Two-phase	35	3	3.48 (0.75)	1.11 (0.12)
LSF	35		4.00	1.00
SSF	35		15.00	3.75

with n = number of references (*jumlah rujukan*)
* = standard deviation (*simpangan baku*)

Intensive systems such as thermophilic semi-continuous and two-phase operation, and the LSF and SSF practised in this study, increase the P substantially. A feasibility study should make clear whether the intensive operation is also more economical. SSF looks very promising although it requires the drying of water hyacinth from a DM of 7.5% to about 30%.

IV. CONCLUSION

Both LSF and SSF systems attain very high production rates in comparison with the Gobar system, popular in tropical countries. This is achieved by intensive and well-managed operation. However, the advantage of LSF is not obvious. Acidification never occurred and hence separation of the methanogenic microbiota doesn't seem to be mandatory.

The high loading rate and productivity made possible by SSF are promising and warrant further research. Indeed, the high return on investment in the reactor must be larger than the expenses and energy required to pretreat the substrate. This should easily be possible by combination of mechanical dewatering, solar drying and eventually waste heat from a generator set.

The most striking outcome of this research is the importance of a constant, mesophilic temperature. Ambient tropical conditions are not enough to reach a steady, high performing digestion. Productivity is at least doubled by working constantly at 35°C.

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