

dioxide. To increase the production of hydrogen, to be assured that organic acids produced as small as possible. To improve the productivity of hydrogen can also be done by controlling the conditions of pH, temperature and HRT (Hydraulics Retention Time) in order to prevent the growth of non hydrogen bacteria culture (Cheng et al., 2011; Liu et al., 2011). Optimal hydrogen production in the fermentation on asidogenesis phase at pH 5.5 and 6.5. At pH 4.5 has begun to form organic acids because *Clostridium acetobutylicum*, *Clostridium butylicum*, and *Clostridium beijerinckii* have the ability to produce ethanol, butanol and acetone at low pH and reduces the formation of hydrogen. Another study using the alkaline conditions in the dark fermentation at pH 10 to avoid the formation of acid and propionate to inhibit the growth of non hydrogen bacteria. It is therefore very important to control the pH of media. Formation of hydrogen gas in fermentation can be carried out under the mesophyll (25-40) °C, thermofil (40-65) °C or hiperthermofil (> 80 °C). The most favorable conditions in the mesophyll because not much need energy ((Claassen et al., 2010; Argun and Kargi, 2011; Sagnak et al., 2011). Isolation of hydrogen producing bacteria like *Bacillus* or *Clostridium* species is essential to increase the productivity of hydrogen. Therefore this study focused on isolating, characterizing and identifying new H₂-producing bacteria from sludge of anaerobic biogas reactor.

MATERIAL AND METHODS

The composition of the culture hydrogen medium (HM) according to the used by reference Stjryanto & Suwanto, 2000; Oztekin et al., 2008; Liu et al., 2010; Ozmihci et al., 2011). The composition of the media can be seen in table 1.

Tabel 1. The composition of hydrogen media (HM).

.No	Stock nutrients	Chemical formula (g/100ml)	Usage (ml/L)
1.	A	NH ₄ Cl (10 g), NaCl (1 g), MgCl ₂ 6H ₂ O (1 g), CaCl ₂ 2 H ₂ O (0,5 g).	10
2.	B	K ₂ HPO ₄ 3 H ₂ O (20 g)	2
3.	C	NaHClO ₃ (10,4 g)	50
4;	D	FeCl ₂ 4H ₂ O (0,2 g), H ₃ BO ₃ (0,005 g), ZnCl ₂ (0,005 g), CuCl ₂ 2H ₂ O (0,0038 g), MnCl ₂ 4H ₂ O (0,005 g), NH ₄ 6 Mo7 (0,005 g), AlCl ₃ (0,005 g), CaCl ₂ 6 H ₂ O (0,005 g), NiCl ₂ 6H ₂ O (0,0092 g), EDTA (0,05 g), Na ₂ SeO ₃ 5H ₂ O (0,01 g), HCl pekat (0,1 ml)	1
5.	E	Yeast ekstrak (10 g)	10
6.	F	Na ₂ S (2,5 g)	10
7.	G	HCl 0,2 N	
8.	H	Glutamic acid (0,001 g), Ascorbic acid (0,0025 g), Riboplavin (0,0025 g), Citric acid (0,002 g), Folic acid (0,001 g), p-amino benzoic acid (0,001 g), Creatine (0,025 g).	1
9.		Glucose	10 g

The stock solutions media were sterilized separate at a temperature of 121° C for 20 minutes. The medium pH is adjusted to be 7 by adding 0.2 M HCl (stock G). Sludge from the anaerobic biogas reactor was wet heat treated (100°C, 2 hrs), cooled and then