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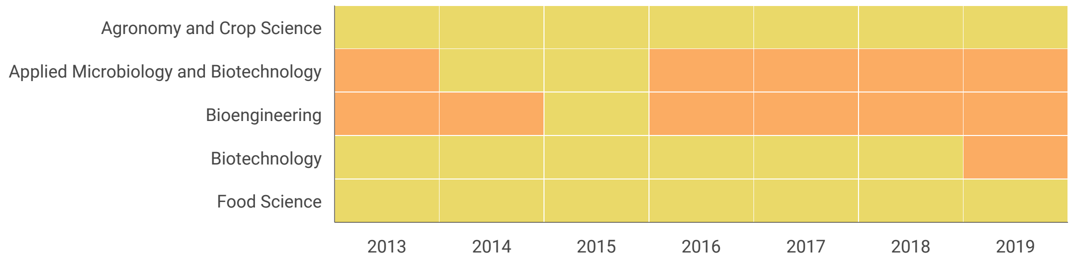

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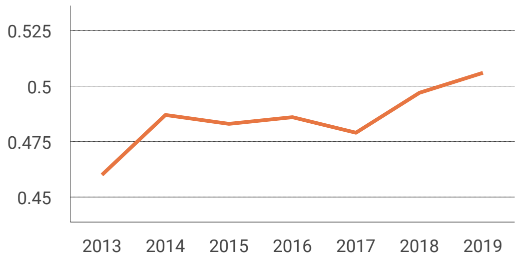

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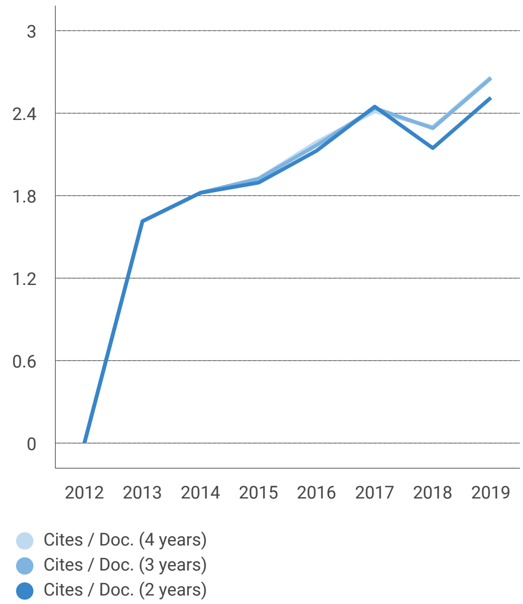

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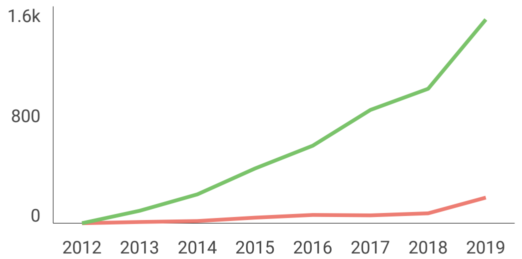

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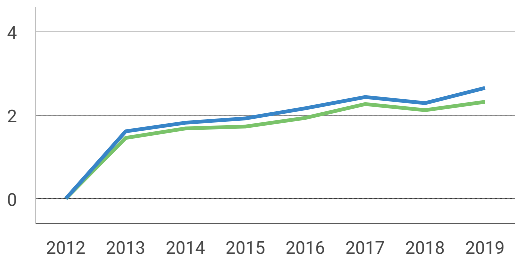

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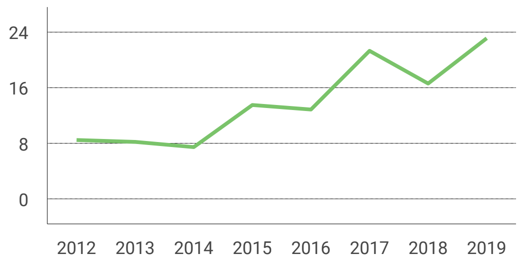

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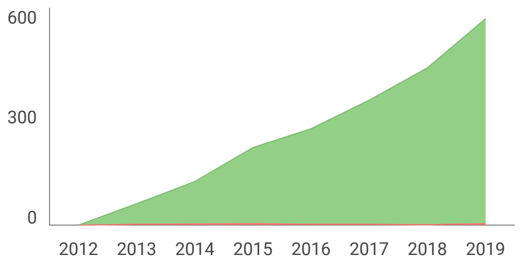

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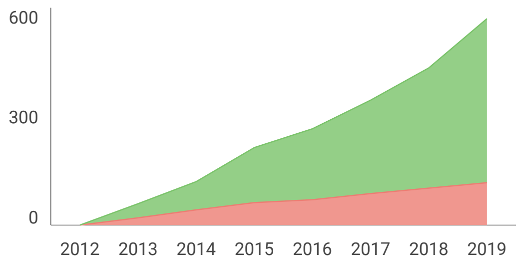

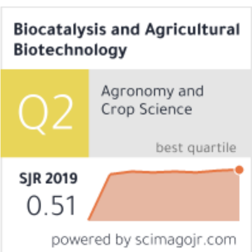

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Bioconversion of agricultural waste hydrolysate from lignocellulolytic mold into biosurfactant by *Achromobacter* sp. BP(1)5

Ni'matuzahroh [a,b,*], Silvia Kurnia Sari [a,b], Nastiti Trikurniadewi [a,b], Syahriar Nur Maulana Malik Ibrahim [a,b], Ana Mariatul Khiftiyah [a,b], Achmad Zainal Abidin [a], Tri Nurhariyati [a,b], Fatimah [a,b]

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ABSTRACT

Rice straw and corn cobs have proven as a promising waste in the bioconversion of biomass into bioproducts. The lignocellulose content found in rice straw and corn cobs has the potential to be hydrolyzed into sugar and used as a carbon source for the growth of microorganisms. This study aims to utilize lignocellulose waste from rice straw and corn cobs for biosurfactant production by *Achromobacter* sp. BP(1)5. Rice straw and corn cobs were hydrolyzed using *Penicillium citrinum* H9 4% (v/v) for 6 days. Sugar content was analyzed using the Somogyi-Nelson method with UV–Visible spectrophotometer. Biosurfactants were produced in synthetic mineral water by adding hydrolysate sugar from rice straw and corn cobs for 7 days and evaluated through measurement of surface tension and emulsification activity. *Achromobacter*'s biosurfactant crude extracts were characterized by critical micelle concentration (CMC) value and stability at the variation in pH, temperature, and salinity. *Achromobacter* sp. BP(1)5 was identified using 16S rRNA. The yields of sugar from rice straw and corn cobs hydrolysis sequentially were 2.260 and 7.880 g/L. The crude biosurfactant from hydrolysate sugar substrate of rice straw and corn cobs had same CMC value that was 6.0 g/L with emulsification activity on kerosene 27.22% and 36.84% respectively. Crude biosurfactant extracts from both substrates were stable on pH 4.0–10.0, temperature 30–70 °C and salinity 0–10% (w/v). This study showed that the agricultural wastes were a cheap material for biosurfactant production, thereby reducing obstacles for biosurfactant production.

1. Introduction

Indonesia is an agrarian country that produces rice and corn in large quantities compared to other agricultural crops. Based on Statistics Indonesia, in Indonesia as many as 75.40 and 19.60 million tons of rice and corn were produced in 2015 (Subagya et al., 2016). Along with the high production of rice and corn, the by-product derived from these two plants, for example corn cobs and rice straw, are also quite abundant in the environment. In Indonesia the use of corn cob and rice straw has not been done much and is less varied, utilization by the community generally only as animal feed and compost.

Rice straw and corn cobs contain lignocelluloses, which consist of lignin, cellulose, and hemicellulose (Shawky et al., 2011; Ghaffar et al., 2017; Mardawati et al., 2018). Several researches proved that lignocellulose biomass from agricultural wastes can be converted into many products (Sunarti et al., 2010; Ghaffar et al., 2017), one of them is into substrates for biosurfactant production (Das and Kumar, 2018; Ni'matuzahroh et al., 2019a; Ni'matuzahroh et al., 2019b).

Biosurfactant is surface-active chemical compounds synthesized by several bacteria and fungi that can be applied in many fields, such as for remediation of oil and heavy metal contaminated sites (Qiao and Shao, 2010; Nwaguma et al., 2016; Gomaa and El-Meihy, 2019; Pele et al., 2019). Biosurfactant is an alternative to non-biodegradable and environmentally harmful surfactants (Moro et al., 2018). Although biosurfactants are save for the environment, biosurfactant production is still expensive (Helmy et al., 2011). Hence many researches has been done to minimize the cost of biosurfactant production by utilizing les valuable raw material (Martins and Martins, 2018; Pele et al., 2019). Some researches showed that agricultural wastes could be the low-cost substrate candidate for biosurfactant production (Moldes et al., 2007;

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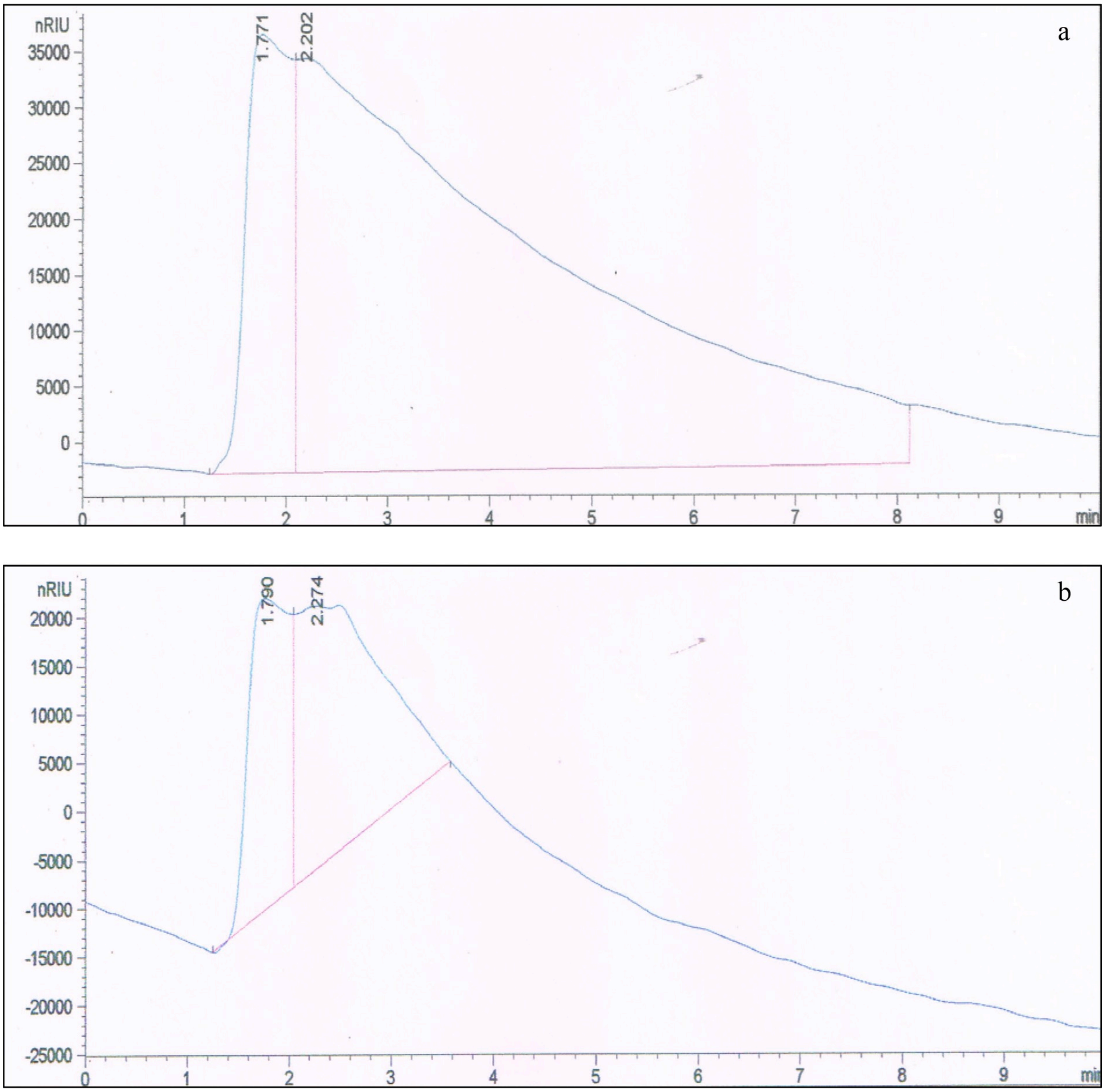

Fig. 1. HPLC chromatogram of agricultural wastes hydrolysate produced by *Penicillium citrinum* sp. H9 from different substrates (a) rice straw and (b) corn cobs.

Ni'matuzahroh et al., 2019a; Ni'matuzahroh et al., 2019b).

Utilization of agricultural waste in bioconversion to biosurfactants involved hydrolysis as pre-treatment for agricultural waste to produce hydrolysate sugar that would be used as a substrate (Moldes et al., 2007). Hydrolysis of agricultural waste could be carried out using enzymes (Boonmee, 2012) and living cell (Ni'matuzahroh et al., 2019b). *Penicillium citrinum* H9 is one of the lignocellulolytic molds that have been known its potency in agricultural waste hydrolysis (Ni'matuzahroh et al., 2019a; Ni'matuzahroh et al., 2019b). As many as 209.25 μg/mL sugar was obtained from the hydrolysis of rice straw by *Penicillium citrinum* H9 (Ni'matuzahroh et al., 2019b).

Besides using low-cost material to overcome the problem in biosurfactant production, another solution is applied good potent biosurfactant producing bacteria (Dos Reis et al., 2018). Hydrocarbonoclastic bacteria, *Achromobacter* sp. BP(1)5 that isolated from Balongan oil sludge was one of the potential bacteria in producing biosurfactant from rice straw hydrolysate than the others isolates (Ni'matuzahroh et al., 2019a). The ability of the bacteria to produce biosurfactant in low-cost substrate indicates the bacteria as a promising isolate for low-cost biosurfactant production. The aims of this research were: to utilize hydrolysate sugar from rice straw and corn cobs through the hydrolysis carried out by *Penicillium citrinum* H9 for biosurfactant production by *Achromobacter* sp. BP (1)5, and to characterize the biosurfactants yielded in each agricultural wastes hydrolysate substrate. Besides that, the identification of the potential bacteria was carried out to reveal another potency that might be had by the bacteria. This research was not only being expected to provide alternatives in biosurfactant production, but also can increase the value of agricultural wastes utilization so that they can be used more widely and reduce the amount of organic waste in the environment.

2. Materials and methods

2.1. Isolate and medium preparation

Penicillium citrinum H9 and *Achromobacter* sp. BP(1)5 are a microbial collection from microbiology laboratory of Biology Department, Universitas Airlangga. *Penicillium citrinum* H9 was re-cultured on Potato Dextrose Agar (PDA) slant and was incubated in room temperature 28 °C for seven days. *Achromobacter* sp. BP(1)5 isolate was grown in nutrient a

Isolate (Taxon)		Taxon / Taxon																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Achromobacter BP1(5)	1		0.077	0.019	0.016	0.019	0.010	0.006	0.006	0.006	0.006	0.006	0.006	0.034	0.034	0.035	0.034	0.031	0.031	0.031	0.031	0.029	0.029
AJ299215.1_Thermoplasma_volcanium_GSS1	2	0.642		0.031	0.030	0.064	0.030	0.030	0.030	0.030	0.030	0.037	0.030	0.028	0.028	0.027	0.034	0.032	0.032	0.031	0.031	0.029	0.029
NR_109523.1_Paenalcaligenes_hermetiae_KBL009	3	0.094	0.578		0.007	0.018	0.007	0.008	0.008	0.008	0.008	0.011	0.008	0.017	0.017	0.017	0.021	0.019	0.018	0.018	0.018	0.013	0.013
MK201766.1_Candidimonas_sp._BO221	4	0.073	0.590	0.069		0.015	0.004	0.005	0.004	0.006	0.005	0.008	0.005	0.018	0.017	0.018	0.020	0.018	0.018	0.018	0.018	0.012	0.013
MH158315.1_Burkholderia_sp._BbQS859	5	0.094	0.649	0.119	0.103		0.016	0.017	0.018	0.017	0.017	0.017	0.018	0.031	0.031	0.030	0.033	0.027	0.028	0.028	0.028	0.025	0.024
NR_160523.1_Orrella_dioscoreae_LMG_29303	6	0.025	0.585	0.082	0.029	0.100		0.004	0.004	0.005	0.004	0.007	0.004	0.017	0.017	0.018	0.020	0.017	0.017	0.017	0.017	0.013	0.013
MK875252.1_Achromobacter_xylosoxidans_IPA-CC9	7	0.010	0.577	0.085	0.032	0.111	0.025		0.002	0.000	0.002	0.002	0.002	0.018	0.018	0.018	0.020	0.018	0.018	0.018	0.018	0.013	0.013
NR_117708.1_Achromobacter_anxifer_LMG_26857	8	0.010	0.583	0.083	0.027	0.114	0.021	0.004		0.003	0.002	0.005	0.002	0.018	0.017	0.018	0.020	0.018	0.018	0.018	0.018	0.013	0.013
MN177171.1_Achromobacter_marplatensis_cjy23	9	0.010	0.592	0.092	0.048	0.111	0.042	0.000	0.021		0.002	0.004	0.003	0.018	0.018	0.018	0.020	0.018	0.018	0.018	0.018	0.013	0.013
MK791143.1_Achromobacter_aegrifaciens_DB58	10	0.010	0.566	0.086	0.031	0.111	0.024	0.005	0.004	0.005		0.002	0.003	0.017	0.017	0.018	0.020	0.018	0.018	0.018	0.018	0.013	0.012
MH074829.1_Achromobacter_insolitus_T3-5-1	11	0.010	0.642	0.119	0.061	0.111	0.047	0.003	0.024	0.019	0.004		0.004	0.023	0.022	0.023	0.027	0.023	0.022	0.022	0.022	0.016	0.016
MK396599.1_Achromobacter_pulmonis_PI3-03	12	0.010	0.584	0.083	0.032	0.114	0.026	0.004	0.005	0.021	0.009	0.021		0.018	0.017	0.018	0.020	0.018	0.018	0.017	0.017	0.013	0.013
AY161046.1_Staphylococcus_cohnii_PLC-9	13	0.271	0.544	0.304	0.318	0.308	0.311	0.310	0.318	0.321	0.305	0.345	0.318		0.001	0.009	0.013	0.009	0.009	0.008	0.008	0.017	0.016
MK208692.1_Staphylococcus_saprophyticus_FC298 1	14	0.271	0.544	0.305	0.318	0.308	0.313	0.309	0.318	0.322	0.304	0.343	0.318	0.001		0.009	0.013	0.009	0.009	0.008	0.008	0.017	0.016
FJ237390.2_Jeotgalicoccus_nanhaiensis_JSM_07702 3	15	0.302	0.517	0.306	0.320	0.273	0.319	0.303	0.324	0.318	0.304	0.339	0.319	0.106	0.105		0.013	0.010	0.009	0.009	0.009	0.017	0.015
DQ466089.1_Bacillus_cereus_SCB001	16	0.283	0.543	0.313	0.301	0.314	0.304	0.305	0.307	0.305	0.309	0.338	0.302	0.134	0.134	0.147		0.009	0.010	0.009	0.009	0.019	0.019
KC527665.1_Bacillus_weihenstephanensis_A2-25c-6b	17	0.260	0.505	0.282	0.262	0.263	0.264	0.267	0.265	0.267	0.267	0.291	0.267	0.078	0.078	0.089	0.072		0.005	0.004	0.004	0.016	0.016
LC189358.1_Bacillus_toyonensis_SBFWS3	18	0.260	0.509	0.276	0.267	0.262	0.266	0.265	0.269	0.265	0.271	0.283	0.265	0.078	0.079	0.085	0.087	0.027		0.003	0.003	0.017	0.016
MK318251.1_Bacillus_paramycoides_NGB-SF327	19	0.260	0.501	0.275	0.265	0.262	0.264	0.264	0.267	0.264	0.270	0.285	0.264	0.070	0.071	0.078	0.074	0.018	0.011		0.001	0.016	0.016
MF372576.1_Campylobacter_jejuni_11643	20	0.260	0.499	0.274	0.264	0.262	0.262	0.263	0.266	0.263	0.268	0.283	0.263	0.069	0.070	0.077	0.074	0.017	0.010	0.001		0.016	0.016
FJ972527.1_Pseudomonas_aeruginosa_CJM	21	0.196	0.567	0.199	0.188	0.214	0.193	0.193	0.197	0.199	0.193	0.241	0.198	0.267	0.266	0.274	0.256	0.220	0.231	0.225	0.224		0.007
FJ972536.1_Pseudomonas_fluorescens_NO7	22	0.199	0.545	0.204	0.211	0.197	0.215	0.194	0.204	0.204	0.188	0.237	0.208	0.262	0.263	0.262	0.237	0.202	0.214	0.208	0.207	0.070	

b

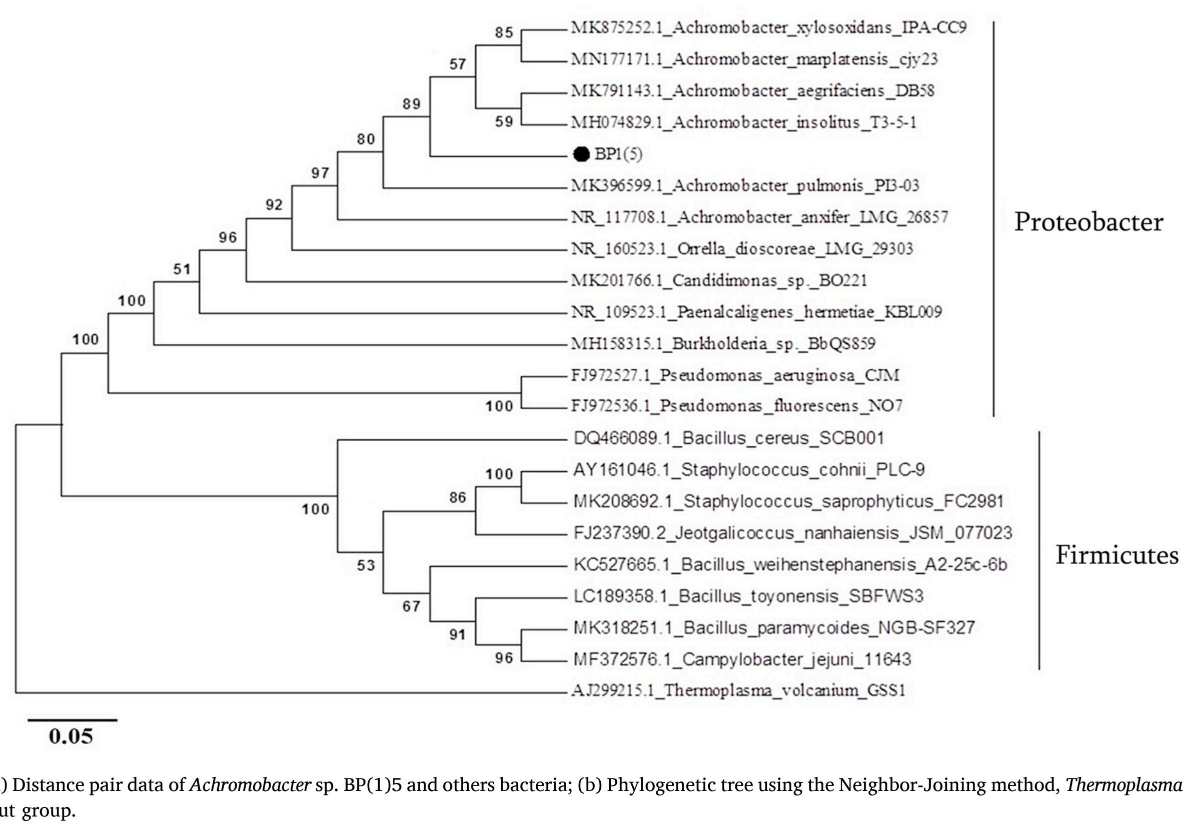

Fig. 2. (a) Distance pair data of *Achromobacter* sp. BP(1)5 and others bacteria; (b) Phylogenetic tree using the Neighbor-Joining method, *Thermoplasma volcanium* was used as out group.

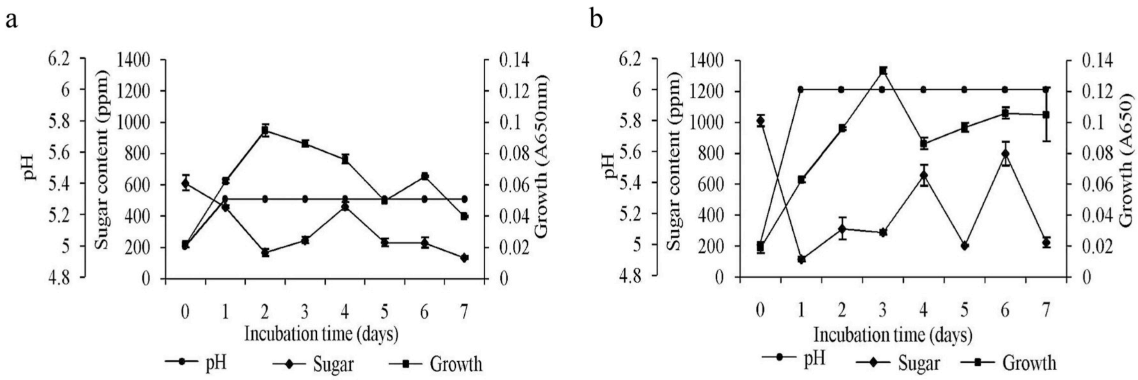

Fig. 3. Growth response of *Achromobacter* sp. BP(1)5 during biosurfactant production for seven days incubation on agricultural waste hydrolisates from different substrates (a) rice straw and (b) corn cobs.

broth medium and incubated using a shaker at 30 °C for 24 h.

The agricultural wastes, rice straw and corn cobs were obtained from Bojonegoro farmer and it were delignified mechanically using grinding and sifted by 40-mesh, then chemically delignified by 1% (w/v) NaOH for 1 h at 100 °C. After delignification, the wastes were washed in water flow until the pH 7 and dried in oven at 60 °C for overnight. Medium that used for saccharification was 100 mL of Mandel Stenberg Mineral (MSM) consisting of 1.4 g/L $(NH_4)_2SO_4$, 2.0 g/L KH_2PO_4, 0.3 g/L $CaCl_2$, 0.3 mg/L $MgSO_4.7H_2O$, 5 mg/L $FeSO_4.7H_2O$, 1.6 mg/L $MnSO_4.H_2O$, 1.4 mg/L $ZnSO_4.7H_2O$, 2 mg/L $CoCl_2$, 2% (w/v) dried substrates and pH 5 that controlled by citric buffer.

Biosurfactant production was carried out on 100 mL of synthetic mineral water (SMW) with the addition of 7.5% (v/v) rice straw and corn cobs hydrolysate in Erlenmeyer flask. SMW used in this study was a modification from Pruthi and Cameotra (1997), the composition of SMW were 3.0 g/L $(NH_4)_2SO_4$, 10 g/L NaCl, 0.2 g/L $MgSO_4.7H_2O$, 0.01 g/L $CaCl_2$, 0.001 g/L $MnSO_4.H_2O$, 0.001 g/L H_3BO_3, 0.001 g/L $ZnSO_4.7H_2O$, 0.001 g/L $CuSO_4.5H_2O$, 0.005 g/L $CoCl_2.6H_2O$, 0,001 g/L $NaMoO_4.2H_2O$, 5 g/50 mL KH_2PO_4, 2.62047 g/50 mL K_2HPO_4, and 0.0006 g/50 mL Fe_3SO_4.

2.2. Rice straw and corn cobs hydrolysis by lignocellulolytic mold

Penicillium citrinum H9 spore was suspended in 10 mL sterile distilled water and measured their turbidity using spectrophotometer $\lambda_{550\ nm}$ to get OD 0.5. After that, 4% (v/v) spore was added in Mandel Stenberg media and incubated for six days, with agitation 120 rpm. The hydrolysate concentration was measured using Somogyi-Nelson method. Rice straw and corn cobs hydrolysate components (glucose, sucrose and fructose) were identified using HPLC Agilent 1100 Series with autosampler, refractive index detective, Agilent Zorbax Carbohydrate column (4.6 × 150 mm, 5 μm), eluent acetonitril:distilled water (75:25) 1.4 mL/min at 30 °C, and sample inject volume 50 μL.

2.3. Identification of Achromobacter isolate using 16S rRNA analysis

Bacterial isolate stock was sub-cultured with three loops colonies in 20 mL of NB medium and incubated in room temperature with agitation of 120 rpm for 48 h. After incubation, isolate was extracted using CTAB method to get the DNA (Ausubel et al., 2003). Concentration and purity of the DNA was carried out using Multiskan GO on $\lambda_{260\ nm}$ and $\lambda_{280\ nm}$. The DNA was mixed with the GoTaq Green Master Mix and 16S rRNA primers 518F (CCAGCAGCCGCGGTAATACG) and 800R (TACCAGGG-TATCTAATCC), then it was amplified using Eppendorf Mastercycler. The conditions of the Polymerase Chain Reaction (PCR) were as follows: initial denaturation of 96 °C for 2 min, denaturation of 96 °C for 45 s, annealing of 51 °C for 30 s, elongation of 72 °C for 2 min, final elongation of 72 °C for 5 min, for 35 cycles. The amplicons were sequenced and analysed their similarity with GenBank data using BLASTn NCBI (Altschul et al., 1997). MEGA 6.0 was used to analyze the distance and to construct the phylogenetic tree (Tamura et al., 2013).

2.4. Biosurfactant production from agricultural waste hydrolysate

Achromobacter sp. BP(1)5 was cultivated in nutrient broth for 24 h. It was measured the optical density until 0.5 at $\lambda_{650\ nm}$. Then, 2% (v/v) of bacterial culture was added on the SMW. Cultures were incubated for seven days with agitation of 120 rpm and temperature of 30 °C. The biosurfactant production was conducted in three replications. During the incubation, every day, the culture was quantified the growth condition, sugar concentration, and pH. After incubation, all cultures were separated between cell and supernatant with centrifugation at 6000 rpm. Precipitation of biosurfactant in the supernatant was carried out by adding of 6 N HCl (v/v) until pH 2 and incubated for a day in 4 °C. The supernatant was centrifuged at 6500 rpm to obtain crude biosurfactant.

2.5. Biosurfactant characterization

Crude biosurfactant was characterized by measuring the critical micelle concentration (CMC) and stability on pH, temperature and salinity, which based on surface tension value and emulsification activity. The CMC value was estimated through evaluation surface tension from crude biosurfactant at concentration 1 g/L to 10 g/L. CMC values were measured at 30 °C and at neutral pH 7. The biosurfactant stability test was done at pH 4, 7 and 10, temperature of 30 °C, 50 °C and 70 °C, and salinity of 0%, 5% and 10% (w/v).

3. Results and discussion

3.1. Hydrolysate product by Penicillium citrinum H9

Enzymatic hydrolysis of rice straw and corn cobs by *Penicillium citrinum* H9 for six days was successfully got 2.260 and 7.880 g/L of reducing sugar. Conversion efficiencies of agricultural waste into hydrolysate were 11.3% and 39.4%, respectively. Retention times of glucose, sucrose and fructose in chromatogram were 2.517, 2.776 and 2.487, respectively. Fig. 1 is chromatogram of rice straw and corn cobs hydrolysate, which the retention times were different from sugars standard. It showed that the type of sugar from rice straw and corn cob hydrolysate were not the third sugar. Meile et al. (2018) investigated the composition of sugars in wood hydrolysate using physical hydrolysis/autohydrolysis, the results showed that lignocellulase was largely converted to xylose.

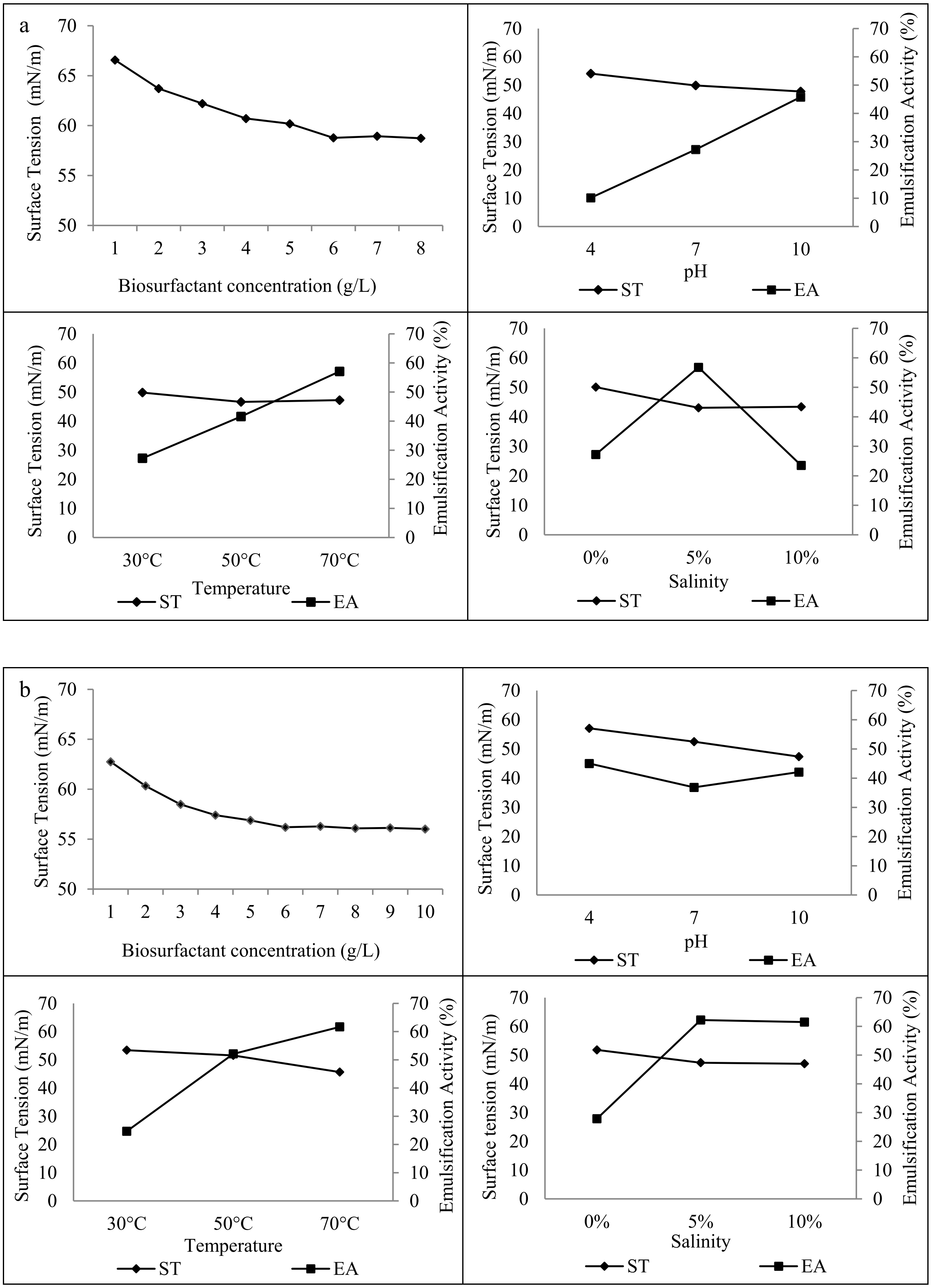

Fig. 4. Characters of *Achromobacter* sp. BP(1)5 biosurfactant on agricultural waste hydrolisates evaluated by Surface Tension (ST) and Emulsification Activity (EA) from different substrates (a) rice straw and (b) corn cobs.

3.2. *Achromobacter sp. BP(1)5 identification based on 16S rRNA*

Achromobacter sp. BP(1)5 was isolated from the Balongan oil sludge (Ni'matuzahroh et al., 2019a). Blast result of 16S rRNA identification approach was *Achromobacter xylosoxidans* IPA-CC9 (GenBank accession no. MK875252) with query cover 98%. The sequence and some related references were analysed using distance method to construct phylogenetic tree (Fig. 2). In distance pair analysis (Fig. 2 (a)), *Achromobacter* sp. BP(1)5 had value 0.010 on taxon no. 7–12, that was genus *Achromobacter*. The lowest value of a distance pair indicates that it is a relative

Table 1
Biosurfactant product of *Achromobacter* in various substrates.

Substrate	Production condition	Yield		CMC		Stability	Reference
		Crude	Purified	Concentration	Surface Tension		
Corn cob + SMW	pH 6.5, 7 days, shaker 120 rpm, 28 °C	0.1 g/L		6000 mg/L	56.2 mN/m	30-70 °C pH 4–7 NaCl 0–10%	This study
Rice straw + SMW	pH 6.5, 7 days, shaker 120 rpm, 28 °C	0.07 g/L		6000 mg/L	58.8 mN/m	30-70 °C pH 4–7 NaCl 0–10%	This study
Glucose (1%) + Yeast Extract (0.05%) + MSM	pH 5.5–7.2, $NaNO_3$ (0.5%), 5 days, shaker 150 rpm, 30-37 °C		Rhamno-lipid	81 mg/L	30.7 mN/m	20-100 °C pH 2–12 NaCl 0–10%	Haloi and Medhi (2019)
Fermentation Medium (g/L): glycerine, 40; urea, 2; yeast extract, 1; KH_2PO_4, 1; $Na_2HPO_4.12H_2O$, 2; $MgSO_47H_2O$, 0.2; NaCl, 10; and the trace element solution (1 ml/L)	150 rev/min, 6 days, 28 °C, pH 7.5	6.84 g/L	0.5 g/L Lipopep-tide	48 mg/L	24.2 mN/m	40-100 °C pH 6–12 NaCl 10–30 g/L	Deng et al. (2016)
Dextrose (3–4% w/v), C/N ratio 8.3 using sodium nitrate and beef extract, 2×10^{-5} g equivalents Fe^{3-}, 1500 mM PO_4^{3-} + MSM	pH 7, 10 days, shaker 120 rpm, 30 °C		4.13 g/L rhamno-lipid	136 mg/L	30.42 mN/m	30–121 °C pH 6–12 0.5–5% w/v	Joy et al. (2019)

taxon, in contrast to the highest value is a distant taxon. Phylogenetic tree from Neighbor-Joining method (Fig. 2 (b)) showed that BP(1)5 is related with *Achromobacter* genera and Proteobacteria phylum. The nucleotide 16S rRNA of *Achromobacter* sp. BP(1)5 has been registered in GenBank with accession no. **MN401689**.

There are many bacteria that can produce biosurfactants, including groups of bacteria in the phylum of Actinobacteria, Firmicutes, and Proteobacteria. Although in the same phylum, each bacterium can produce different types of biosurfactants. The biosurfactant product from Actinobacteria phylum such as *Rhodococcus*, *Nocardia*, *Gordona*, *Mycobacterium*, *Corynebacterium*, and *Micrococcus*, are classified as trehalolipid, a glycolipid biosurfactant type (Kim et al., 2000; Franzetti et al., 2009; Tuleva et al., 2009; Ivanova et al., 2016; Dwivedi et al., 2019; Kuyukina and Ivshina, 2019). *Arthrobacter* produced arthrofactin, a lipopeptide biosurfactant type, and *Actinomyces* and *Streptomyces* that are still rare identified their biosurfactant type (Morikawa et al., 1993; Thampayak et al., 2008; Olajuyigbe and Ehiosun, 2016). Likewise with the Firmicutes phylum, biosurfactant from *Clostridium* has not been completely identified the type of biosurfactant, *Lactobacillus* was produced glycoprotein, a high molecule biosurfactant. *Bacillus* and *Staphylococcus* produces lipopeptide biosurfactant. (Cooper et al., 1980; Banat et al., 2010; Eddouaouda et al., 2012; Madhu and Prapulla, 2013). Bacteria in Proteobacteria phylum also produce biosurfactant. Biosurfactant from *Pseudomonas and Alcanivorax* are rhamnolipid (glycolipid biosurfactant type), and *Acinetobacter* produce emulsan and biodispersant (high molecule biosurfactant) (Rosenberg et al., 1988; Abraham et al., 1998; Lang and Wullbrandt, 1999; Banat et al., 2010; Ohadi et al., 2017). Burkholderiales order has some genus, for instance, *Burkholderia*, *Paraburkholderia*, *Bordetella*, and *Achromobacter* that have different biosurfactant product, which are rhamnolipid (glycolipid biosurfactant type) from *Burkholderia* and *Paraburkholderia*; lipopeptide and glycolipid biosurfactant type from *Achromobacter*; and unidentified biosurfactant from *bordetella* (Tavares et al., 2012; Odukkathil and Vasudevan, 2015; Deng et al., 2016; Joy et al., 2019).

Genomic of *Achromobacter* had revealed by Hong et al. (2017), from *Achromobacter* sp. HZ01 was found biosurfactant genes, which are LuxR family transcriptional regulator gene that important to synthesize glycolipid and one gene as non-ribosomal peptide synthetases that indicated to produce lipopeptide, then it proved by lipopeptide structure that got from *Achromobacter* sp. HZ01 (Deng et al., 2016). By those references, it can be estimated if *Achromobacter* sp. BP(1)5 can produce glycolipid and lipopeptide biosurfactants.

3.3. Production and characterization of Achromobacter sp. BP(1)5 biosurfactant

The growth of BP(1)5 increased on the first day and has entered the death phase on the 7th day of incubation. Based on Fig. 3, there is sugar utilization by BP(1)5 for its growth. Sugar concentration decreased from 1.010 g/L up to 0.223 g/L on corn cobs hydrolysate substrate and 0.611 g/L to 0.135 g/L on rice straw hydrolysate for 7 days incubation. The pH was measured during the incubation of BP(1)5 isolate. On the corn cobs hydrolysate substrate, the pH range of culture tends to be 5.0–6.0 and on the rice straw hydrolysate substrate 5.0–5.3.

Yañez-Ocampo et al. (2017) has found that the increasing sugar concentration in final day incubation of biosurfactant production by bacteria in cooking oil waste and coffee waste was inversely proportional with the first phase incubation that has low concentration. It was due to the bacteria produced glycolipid biosurfactant, which related with the biosurfactant product from *Achromobacter*. Consumption of sugar by bacteria was accompanied by the formation of biosurfactant products. Joy et al. (2019) stated that *Achromobacter* grown on MSM media with the addition of lignocellulosic-rice straw hydrolysate resulted in the amount of rhamnolipid which was almost similar to when the isolates grown on chemically defined medium which had a total-sugars composition 4.55% consist of glucose 2.8%, cellobiose 0.14%, xylose 1.5% %, and arabinose 0.11%. This shows that the composition of hydrolysate sugar of rice straw does not differ greatly from the composition of sugar in chemically defined media.

Biosurfactant product was harvested at 7th day, which products obtained as much as 0.074 g/L in rice straw hydrolysate and 0.095 g/L in corn cobs hydrolysate. Bioconversion percentages from rice straw and corn cobs hydrolysate into biosurfactants were 12.1% and 9.4% respectively. The percentages were not as good as expected. This can occur because the hydrolysate sugar is largely inaccessible to bacteria to be converted into biosurfactants. The main point of the result is *Achromobacter* sp. BP(1)5 can use low-cost substrate to produce biosurfactant, but further research is still needed to optimize hydrolysis of agricultural waste to obtain the most suitable substrate for biosurfactant production using *Achromobacter* sp. BP(1)5 and biosurfactant production of *Achromobacter* sp. BP(1)5.

The surface tension activity at critical micelle concentration value of corn cobs hydrolysate was lower than rice straw hydrolysate. CMC value on corn cobs hydrolysate substrate was 6 g/L with a surface tension reduction value of 56.2 mN/m, while CMC value on rice straw hydrolysate substrate was 6 g/L with a value of surface tension reduction of

58.8 mN/m. Fig. 4 shows the critical micelle concentration value and stability test on pH, temperature and salinity of biosurfactant product.

Biosurfactant of BP (1)5 still has activity to reduce surface tension and emulsification under pH 4.0–10.0, temperature 30 °C - 70 °C, and salinity 0%–10% (w/v). The higher temperature, pH, and salinity tend to decrease surface tension activity, whereas the higher temperature and pH increase the emulsification activity value, the value of emulsification activity tends to decrease at salinity concentrations of more than 5% (w/v) on both substrates.

Measurement of the stability of biosurfactant products was carried out to determine the prospects for application of biosurfactant products. *Achromobacter* is known to be able to produce biosurfactants on various substrates. Table 1 is a comparison of biosurfactant data produced by *Achromobacter* by other researchers.

Agricultural wastes could be hydrolyzed into reducing sugars. The reducing sugars were converted by microbes into biosurfactant products. Biosurfactant production cost using the hydrolysate sugar substrate was hopefully cheaper than commercial sugar, but it still requires optimization of production conditions to obtain a greater yield. Based on Table 1, on different types of substrates, *Achromobacter* could produce different types of biosurfactants (glycolipids or lipopeptides), but the type of biosurfactant produced by *Achromobacter* sp. BP(1)5 have not been analysed yet. Further research is needed to reveal the types and coding genes of biosurfactants produced by *Achromobacter* sp. BP(1)5.

4. Conclusion

The hydrolysate sugar of rice straw and corn cobs by *Penicillium citrinum* H9 could be converted to biosurfactants by *Achromobacter* sp. BP(1)5. Based on 16S rRNA analysis, *Achromobacter* sp. BP(1)5 had query cover 98% with *Achromobacter xylosoxidans*. Crude extract of *Achromobacter* sp. BP(1)5 biosurfactant produced on the hydrolysate substrate from rice straw and corn cobs had the same CMC value of 6.0 g/L with emulsification activity on kerosene 27.22% and 36.84% respectively. The biosurfactant crude extracts from both substrates were relative stable at variation of pH, temperature, and salinity.

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Appendix A. Supplementary data

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