The Effect of COD:N Ratios and pH Control to Biogas Production from Vinasse

S. Sumardiono, I. Syaichurrozi*, Budiyono, S. B. Sasongko

Department of Chemical Engineering, University of Diponegoro Postal Code 50239,
Semarang, Indonesia

ABSTRACT

Aims: Vinasse is bottom product of distillation unit from alcohol industry contains Chemical Organic Compound (COD) in high concentration. The purpose of this study was to study the effect of COD:N ratios of substrate and pH control to biogas production from vinasse.

Study Design: This study used anaerobic digestion-laboratory scale at room temperature in batch system. Urea was added as nitrogen source to adjust COD:N ratios of 400:7, 500:7, 600:7, and 700:7. Initial pH for all variables was adjusted 7.0 by using NaOH solution.

Place and Duration of Study: Waste-treatment Laboratory, Department of Chemical Engineering, University of Diponegoro, Indonesia, between August 2012 and January 2013.

Methodology: Vinasse used obtained from the alcohol industry that produced alcohol from molasses. Polyethylene bottles which had volume 5 liters were used as digester. Vinasse of 1 liter was put into digester. Urea was added to make various of COD:N ratio. Initial pH of all variables was adjusted 7.0 by using 10 N NaOH solution. Rumen fluid (10%v/v vinasse) was added as methanogenic bacteria inoculum. Biogas formed was measured by using water displacement method every once in two days to know biogas production daily. pH of substrates in the digesters was measured by using pH meter every once in two days to know pH profile daily. COD of substrates was measured by using COD meter. Solution of NaOH 2N was used to maintain pH of substrate in range neutral condition (7.0±0.2) during the fermentation process.

Results: Biogas formed at COD:N ratio of control variable (1436:7); 400:7; 500:7; 600:7; 700:7 were 3.673; 4.909; 6.079; 6.096; 5.630 mL/g COD, respectively. pH profiles for all variables were decreasing from beginning until ending of fermentation. With controlled pH, pH of substrates was maintained at neutral condition, so methanogenic bacteria could grow well in the digesters. Consequently biogas formed at controlled pH was larger than that at uncontrolled pH. The values of COD removal for COD:N ratio of control variable; 400:7; 500:7; 600:7; 700:7 were 1.27±0.43; 1.59±0.43; 2.85±0.39; 3.21±0.49; 2.22±0.39%, respectively at uncontrolled pH, whereas at controlled pH the values of that were 11.98±0.56; 12.82±0.56; 12.03±0.94; 13.05±0.35; 12.61±0.56 %, respectively.

Conclusions: COD:N ratios of 400:7, 500:7, 600:7, 700:7 produced more total biogas than control variable. Variable of COD:N of 600:7 generated the most total biogas which was 6.096 mL/g COD. Biogas production on pH control was greater than that on pH non-control. At non-controlled pH, COD:N ratio of 600:7 had COD removal 3.21±0.49%. Whereas at controlled pH, COD:N ratio of 600:7 had COD removal 13.05±0.35%.

Keywords: biogas, COD:N ratio, pH control, vinasse

1. INTRODUCTION
Molasses is the residue remaining of the sugar crystallization process that contains 52% of fermentable sugars [1]. Because of its contents, molasses can be used as substrate for fermentation process. In the chemical industry, molasses the most widely is used to produce alcohol in the alcohol industry [2].

Alcohol is produced by fermentation yeast from molasses. Alcohol formed must be separated from fermentation broth by distillation. The bottom product of distillation process is residue known as vinasse. Vinasse is typical of liquid waste that has the relatively high values of chemical oxygen demand, more than 100,000 mg/L [3-4]. As a consequence, vinasse will contaminate the environment, if it be discharged directly into the environment [2,5]. The production of 1-liter alcohol generates 8 - 15 liters of vinasse [2-3,6-7].

Vinasse must be treated first before it is released into the environment because of its COD contents. Aerobic treatment has some shortages, which are extensive land requirement, capital high cost, and operating high cost. On the other hand, Anaerobic treatment can produce energy and be non-polluting [8]. In anaerobic treatment, various type of biomass are decomposed to biogas by microbial activities. This treatment uses digester to transform COD of biomass into biogas at the anaerobic condition [9]. Biogas is sustainable energy source that contains 50-75% methane gas, 30-50% carbon dioxide, small amounts of other gases (CO, N₂, H₂, H₂S, O₂), depending on the substrate [10-12]. So, anaerobic treatment provides method for disposal of wastes and produce biogas as renewable energy.

Vinasse has potential to be processed into biogas. The high COD of vinasse can be destroyed by methanogenic bacteria in the digester. Some authors studied treatment of vinasse in the anaerobic digestion. Espinoza-Escalantea et al. [13] and Budiyono et al. [14] studied the effect of pH to biogas production. Biogas production at pH 6.5 was greater than that at pH 4.5 and 5.5. Buitron and Carjaval [15] studied effect of temperature of anaerobic digestion to biogas production. Biogas production at 35°C was greater than that at 25°C. Siles et al. [16] reported that ozonation pre-treatment affected the methane yield coefficient of 13.6% and the methane production rate with ozonation pre-treatment 41.6% as much as that without ozonation pre-treatment. However, this process required expensive installation costs.

Previous research did not study the effect of COD:N ratios of substrate to biogas production from vinasse yet. According to Speece [9], wastewater containing COD will be destroyed and produced biogas optimally if substrate contains COD:N ratios in the range of 350:7 – 1000:7. While Lutoslawski et al. [4] reported that rate of organic material degradation by microorganisms at pH control was faster than that at pH non control. So, the objective of this study was investigation the effect of COD:N ratios of substrate and pH control to biogas production from vinasse.

2. MATERIALS AND METHODS

2.1 Wastewater and Inoculum

The wastewater used was vinasse obtained from alcohol industry located in Solo, Central Java, Indonesia, that produced alcohol from molasses. The vinasse characteristic used in this study can be seen in Table 1. The rumen fluid was used as inoculum (methanogenic bacteria provider). In this study, rumen fluid that was in fresh condition was obtained from slaughterhouse in Semarang, Central Java, Indonesia.
Table 1. Vinasse characteristic

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>299,250±1,060,660</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>268,647±0.000</td>
</tr>
<tr>
<td>Protein</td>
<td>9,117±0.000</td>
</tr>
<tr>
<td>TS</td>
<td>27.94±0.085</td>
</tr>
<tr>
<td>pH</td>
<td>3.25±0.212</td>
</tr>
<tr>
<td>N</td>
<td>1,458±0.000</td>
</tr>
<tr>
<td>COD:N</td>
<td>1436:7</td>
</tr>
</tbody>
</table>

Remarks: COD, chemical oxygen demand (mg/L); TS, total solid (%); pH, power of hydrogen; N, nitrogen content (mg/L); Carbohydrate (mg/L); Protein (mg/L).

2.2 Experimental set up

Anaerobic digesters of experimental laboratory were made from polyethylene bottles which had a volume of 5 liters. These bottles were plugged with rubber plug and were equipped with valve for biogas measurement. Anaerobic digesters were operated in batch system and at room temperature. Biogas formed was measured by using liquid displacement method as also has been used by the other authors [17-19]. The schematic diagram of experimental set up is shown in Fig. 1. This study were performed in ten variables (Table 2.), each variable was repeated two times using two digesters having the same contents, so this study needed 20 digesters.

![Figure 1. Anaerobic digestion of experimental laboratory](image)

2.3 Experimental design

2.3.1 Study of COD:N ratios

Anaerobic digestions of experimental laboratory using 5-liter volumes were operated in batch system. 1-liter vinnase was put into the digester. Rumen fluid as methanogenic bacteria provider was added into the digester as much as 10% v/v vinasses. From Table 1, can be known that COD:N ratio of vinasse was 1436:7. Whereas ratio COD:N is optimum to produce biogas on range of 350:7 – 1000:7 [9]. Furthermore, urea as nitrogen source was added into the digester to adjust COD:N ratios of 400:7, 500:7, 600:7, 700:7. Initial pH for all variables was adjusted 7.0 by using NaOH solution 10 N. The variables in this study can be seen in Table 2 (a).
Table 2. Variation of pH treatment and COD:N ratios

<table>
<thead>
<tr>
<th>Condition of pH</th>
<th>COD:N substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Uncontrolled</td>
<td>1436:7 (Control-without urea addition)</td>
</tr>
<tr>
<td></td>
<td>400:7</td>
</tr>
<tr>
<td></td>
<td>500:7</td>
</tr>
<tr>
<td></td>
<td>600:7</td>
</tr>
<tr>
<td></td>
<td>700:7</td>
</tr>
<tr>
<td>(b) Controlled</td>
<td>1436:7 (Control-without urea addition)</td>
</tr>
<tr>
<td></td>
<td>400:7</td>
</tr>
<tr>
<td></td>
<td>500:7</td>
</tr>
<tr>
<td></td>
<td>600:7</td>
</tr>
<tr>
<td></td>
<td>700:7</td>
</tr>
</tbody>
</table>

2.3.2 pH control to biogas production

In this study, pH initial for all variables was adjusted 7.0 by using NaOH 10 N before rumen fluid was added. Anaerobic digesters (volume 5 L) were operated in batch system. Volume of 1-liter vinasse and 10% v/v vinasse of rumen fluid were put into digesters. Furthermore, urea as nitrogen was added into the digester to adjust the COD:N ratios (400:7, 500:7, 600:7, 700:7). In this study, pH was controlled constant (pH 7.0±0.2) during biogas formed by using NaOH solution 2 N (Table 2.(b)). NaOH solution 10 N was used by authors to adjust initial pH because characteristic of vinasse was very acid. Using NaOH with high concentration (10 N) can avoid the addition of total volume in the digester. Whereas, during fermentation NaOH 2 N was used to control pH at range 7.0±0.2 because the high concentration of NaOH (more than 2 N) can kill bacteria in the digesters.

2.3.3 COD removal

Chemical Oxygen Demand (COD) was converted by bacteria to biogas at anaerobic condition in the digester [9]. Influent COD and effluent COD of substrates were measured by using COD meter. COD of influent substrates and COD of effluent substrates were used to calculated COD removal (%).

\[
\text{COD removal} \% = \left( \frac{\text{Influent COD} - \text{Effluent COD}}{\text{Influent COD}} \right) \times 100\%
\]

2.4 Experimental procedures

Biogas formed was measured once in two days by using water displacement method (Fig. 1). pH of substrate in the digesters was measured by using pH meter once in two days to know pH profile daily. COD of substrates was measured by using COD meter. At the non-controlled pH, fermentation process was done in the digester until biogas run out to produce. While at the controlled pH neutral, fermentation process was done for 90 days.

3. RESULTS AND DISCUSSION

3.1 Effect of COD:N ratio to Biogas Production

COD is amount of oxygen needed for waste material in the water that can be oxidized through a chemical reaction. COD:N ratio of substrate is necessary parameter to produce biogas optimally. The COD:N range of 350:7 – 1000:7 is the optimal range for anaerobic digestion [9]. If more or less than the range, microbial growth in the digester will be hampered. So, adjustment of COD:N of substrate was needed to be done. In this
experiment, vinnase obtained had COD:N = 1436:7 consequently nitrogen source must be added into the substrate. Proteins, amino acids and urea are nitrogen source needed by microbe to build cell structures [20-21]. For this experiment, urea ([NH_2]_2CO) was added as nitrogen source.

From Fig. 2., COD:N ratios of 400:7 – 700:7 generated more total biogas than control variable (COD:N = 1436:7). This result corresponded with Speece’s statement [9] that substrate with COD:N ratio more than 1000:7 will produce biogas in little amount. Biogas produced at COD:N ratios of 500:7 and 600:7 were in nearly equal numbers. Whereas, biogas produced at COD:N ratio of 400:7 was less than that of 500:7, 600:7, and 700:7. Control, 400:7, 500:7, 600:7, 700:7 variables had total biogas production of 3.673, 4.909, 6.079, 6.096, 5.630 ml/g COD respectively. Among the five variable in various COD:N ratios, COD:N ratio = 600:7 had the largest total volume of biogas and control variable had the least total volume of biogas of the all variables (Fig. 2. (b)).

Protein and urea were decomposed into ammonia/ammonium. Ammonia (NH_3)/ammonium (NH_4^+) was used by bacteria as a nitrogen source [22]. However, the ammonia/ammonium would be toxic for bacteria if the amount of that was too much [23]. De-Baere et al. (1984) reported that concentration of ammonia 100-140 mg/L hampered the bacterial growth at mesophilic temperature. Bacteria could not thrive in substrate that contained ammonia concentrations above 200 mg/L [24]. Methanogen bacteria was the least tolerant and the most easily killed to ammonia inhibition among the four anaerobic microorganisms in four step biogas production there were hydrolysis, acidogenesis, acetogenesis, methanogenesis [25]. Changing ammonia into ammonium was depended on pH condition. Ammonium was less toxic than ammonia. Ammonium disturbed bacterial activity just in high concentration. Concentration of ammonium of 1,500-10,000 mg/L was inhibition start for bacterial growth, whereas that of 30,000 mg/L was toxicity concentration [26].
Figure 2. Biogas production daily (a), biogas production cumulative (b), pH profile (c) at various ratio of COD:N – uncontrolled pH

Chen et al. [27] reported that COD:N ratio of 70 has been suggested for the stable performance of anaerobic digestion. This report could approach with the result of this study, which the range of COD:N ratios of 71.4 – 85.7 (500:7 – 600:7) showed the satisfactory results. In control variable, availability of nitrogen source was too few (Table 1.). Hence, bacteria in the digester could not build cell structures well and the rate biogas production was low (Fig 2. (a)).

Mata-Alvarez et al. [28] reported that carbohydrate-rich substrates were good producers of VFAs and protein-rich substrates were good buffering capacity due to production of ammonia. So, if ratio between carbohydrate (C) and protein (N) are appropriate, biogas will be produced optimally. The value of C (carbon) can be obtained from COD where C = COD value × (12/32) [29]. Vinasse used in this experiment contained large amounts of carbohydrate and small amount of protein (Table 1.). During the fermentation process, VFAs was formed in large quantities and pH substrates dropped directly (Fig. 2.(c)) Elbeshbishy.
and Nakhla [30] also reported that decreasing in the pH could be due to the rapid VFAs production at the beginning, while the increasing in the pH from 3rd day to 10th day could be due to generation of NH4–N during protein degradation, as ammonia which was a base combines with carbon dioxide and water to form ammonium bicarbonate, a natural pH buffer. In this experiment, pH was decreasing from beginning until ending of fermentation (Fig 2(c)). This phenomenon was caused by very large accumulation of VFAs production in the digester. Condition of pH in the substrates which was very acid caused death of methanogenic bacteria. Ammonia generated by degradation urea and protein could not increase the pH because VFAs was produced rapidly.

3.2 Effect of pH control to Biogas Production

At controlled pH, biogas formed was greater than that at uncontrolled pH. Biogas was produced until 90th day and might still be produced until organic matter in the substrate discharged completely (Fig 3.). The comparison total biogas between at pH control and pH non-control can be seen in Fig. 4. The influence of changing in pH was very sensitive to fermentation process that was done by bacteria activity. Therefore, pH control was important parameter for application of biogas production [4,9]. The drop of pH was caused acidogenic bacteria produced acetate, hydrogen gas, carbon dioxide, and few other VFA such as propionic and butyric acid. A low of pH value inhibited the activity of microorganisms involved in the biogas production especially methanogenic bacteria [9,31].

The neutral pH characterize was optimal condition in anaerobic biotechnology. Low pH condition might be caused by two sources of acidity (H2CO3 and VFA), which were generated by bacterial activity. These acids required alkalinity for neutralization so that the bacterial activity was not hindered by pH depression [9]. According to Ayu and Aryanti [32], to allow the growth of methanogenic bacteria, digester should be properly fed and buffered to rising alkalinity. Sodium carbonate (Na2CO3) could increase alkalinity or buffering capacity of fermenting slurry to control pH substrate. Viswanath et al. [33] mentioned that there was a perfect link between the acidogenesis and methanogenesis phases when the pH was remained at 7.0 and there was no drastic increase in acidity or alkalinity. According to Lutoslawski et al. [4], pH neutralization could be done by addition of H2SO4 and NaOH to maintain constant of pH in neutral condition. Biodegradation at pH control caused the final number of bacterial cell total in the digester was more than in process at pH non-control, so that degradation rate of organic matter at pH control was faster than that at the other one.

With pH control, biogas production daily and biogas production cumulative were more than that at pH non-control (Fig 4.). Methanogens prefer nearly neutral pH conditions with generally accepted optimum range of approximately 6.5-8.2 and biogas production rate will decline at pH condition either higher or lower [9]. Hence, if pH condition was maintained, methanogens would grow well and produce biogas in large amount. Total biogas at controlled pH of control, 400:7, 500:7, 600:7, 700:7 variable were 17.875, 20.385, 18.060, 21.229, 18.504 mL/g COD respectively.
Organic contents in the substrate will be destroyed to be biogas. Running out biogas production was caused two things. First, methanogenic bacteria in the digester cannot grow well and finally death. This phenomenon can occur if condition inside the digester does not support the growth of methanogenic bacteria such as temperature, pressure, nutrition, and pH condition. At non-controlled pH, pH of all variables was decreasing until pH 3.0 (Fig. 2(c)). This condition caused death of methanogenic bacteria. Second, organic contents or COD of substrate is exhausted. This phenomenon can occur if condition of digester is not to be limiting parameter. Organic contents or COD are destroyed by microorganisms into biogas. At controlled pH, pH condition of substrates was maintained at 7.0±0.2, so pH was not a limiting parameter and microorganisms in the digesters could grow well (Fig. 3.).

From Fig. 3, biogas production did not stop yet after 90 days observation. Biogas might be still produced until organic contents were completely discharged. Budiyono et al. [18-19] reported that after 90 days cattle manure in the anaerobic digester still produced biogas. This phenomenon showed that organic contents of cattle manure did not exhausted yet and microorganisms still destroyed organic contents actively. Whereas, Lopes et al. [34] state that organic content of substrate did not completely after 360 days observation yet.

Figure 3. Biogas production daily (a), biogas production cumulative (b), at various ratio of COD:N - controlled pH
Speece [9] stated that 1 gram COD can convert into 395 mL biogas at room temperature. In this study, maximum biogas formed was 21.229 mL/g COD. This result was very far from the Speece’s estimation. This result also was lower than other author’s results (Table 3.). The differences in results might be related to differences in amount of organic material in the substrates. According to Soeprijanto et al. [35], biogas formed very influenced by the amount of organic materials in the initial substrates. Besides that, organic material which was too large in the substrates caused overloading in the digesters [18]. From Table 3., vinasse used in this study had high COD content and this value was larger than that in the other studies. Overloading in organic material caused the slowly destroyed organic material rate that was done by bacteria, so the rate of biogas formation was being slowly and total biogas formed was just little in amount.

Table 3. Comparison of result between this study and other studies

<table>
<thead>
<tr>
<th>Influent COD (g/L)</th>
<th>Volume (mL/g COD)</th>
<th>COD:N</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>43.36(^a)</td>
<td>-</td>
<td>[13]</td>
</tr>
<tr>
<td>66.10</td>
<td>284(^a)</td>
<td>-</td>
<td>[16]</td>
</tr>
<tr>
<td>5</td>
<td>110(^a)</td>
<td>500:5</td>
<td>[35]</td>
</tr>
<tr>
<td>35 - 45</td>
<td>252(^b)</td>
<td>-</td>
<td>[36]</td>
</tr>
<tr>
<td>61.2</td>
<td>330(^a)</td>
<td>-</td>
<td>[37]</td>
</tr>
<tr>
<td>299.25</td>
<td>21.23(^b)</td>
<td>600:7</td>
<td>This study</td>
</tr>
</tbody>
</table>

Remarks: \(a = CH_4\) volume (mL/g COD), \(b = \) biogas volume (mL/g COD)

3.3 COD Removal

Organic materials (COD) of substrates will be converted into biogas by activities of bacteria. These activities consist of four levels which are hydrolysis, acidogenesis, acethogenesis and methanogenesis. Amount of COD that is degraded by bacteria in the digester is COD removal [9]. In this study, COD substrate for all variables decreased at the end of fermentation process. Value of COD removal for all variables can be seen in Table 4.
At non-controlled pH, COD removal of 600:7 was the most of all variables which was 3.21±0.49%, then followed by 500:7, 700:7, 400:7 and control variable (Table 4). From Fig. 4., the sequence of variables that generate biogas from the most to the less total biogas was 600:7, 500:7, 700:7, 400:7 and control variable. From this result, can be concluded that the more COD was removed, the more biogas was produced.

COD removal at controlled pH was bigger than at uncontrolled pH condition. Lutoslawki et al. [4] stated that degradation rate of organic contents would do fast, if pH condition of substrate was adjusted neutral. Variable with COD:N ratio of 600:7 had COD removal value which was the most of all variables. From Fig. 4., the most of total biogas production was 600:7. This result similar with Speece’s statement [9] and Soeprijanto et al. [35] that biogas formed would be comparable to the number of missing COD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>COD:N</th>
<th>pH condition</th>
<th>Influent COD (gram)</th>
<th>Effluent COD (gram)</th>
<th>COD removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>300.85±1.06</td>
<td>297.02±0.38</td>
<td>1.27±0.43</td>
</tr>
<tr>
<td>400:7</td>
<td>Uncontrolled</td>
<td>300.85±1.06</td>
<td>296.06±0.38</td>
<td>1.59±0.43</td>
<td></td>
</tr>
<tr>
<td>500:7</td>
<td>Uncontrolled</td>
<td>300.85±1.06</td>
<td>292.27±0.00</td>
<td>2.85±0.39</td>
<td></td>
</tr>
<tr>
<td>600:7</td>
<td>Uncontrolled</td>
<td>300.85±1.06</td>
<td>291.19±0.76</td>
<td>3.21±0.49</td>
<td></td>
</tr>
<tr>
<td>700:7</td>
<td>Uncontrolled</td>
<td>300.85±1.06</td>
<td>294.17±0.00</td>
<td>2.22±0.39</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Controlled</td>
<td>300.85±1.06</td>
<td>264.80±1.13</td>
<td>11.98±0.56</td>
<td></td>
</tr>
<tr>
<td>400:7</td>
<td>Controlled</td>
<td>300.85±1.06</td>
<td>262.29±1.13</td>
<td>12.82±0.56</td>
<td></td>
</tr>
<tr>
<td>500:7</td>
<td>Controlled</td>
<td>300.85±1.06</td>
<td>264.65±2.25</td>
<td>12.03±0.94</td>
<td></td>
</tr>
<tr>
<td>600:7</td>
<td>Controlled</td>
<td>300.85±1.06</td>
<td>261.58±0.00</td>
<td>13.05±0.35</td>
<td></td>
</tr>
<tr>
<td>700:7</td>
<td>Controlled</td>
<td>300.85±1.06</td>
<td>262.91±1.13</td>
<td>12.61±0.56</td>
<td></td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

Vinnase obtained had COD:N = 1436:7. Consequently urea addition was necessary to do. Variable with COD:N ratio of 600:7 had total biogas production of 6.096 mL/g COD which was the most of all COD:N variables. Control variable (without urea addition) had total biogas production was the least of all variables which was 3.673 mL/g COD. Biogas production with controlled pH was greater than that with uncontrolled pH. At controlled pH, total biogas maximum was 21.229 mL/g COD that was produced at COD:N ratio of 600:7. From this study, the optimal condition was substrate with COD:N ratio of 600:7 either at uncontrolled or controlled pH. There was proportional relationship between COD was removed and biogas was produced. The more COD was removed, the more biogas was generated. At uncontrolled pH, COD:N ratio of 600:7 had the value of COD removal of 3.21±0.49%. Whereas at controlled pH, COD:N ratio of 600:7 had the value of COD removal of 13.05±0.35%.

ACKNOWLEDGMENTS

The authors thank University of Diponegoro and Indonesian Ministry of Higher Education via Hibah Kompetensi 2013 grant for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
AUTHORS’ CONTRIBUTIONS

‘Author 1’ and ‘Author 2’ designed the study, wrote the protocol, and wrote the first draft of the manuscript. ‘Author 1’, ‘Author 3’ and ‘Author 4’ managed the analyses of the study. ‘Author 2’ managed the literature searches. All of authors read and approved the final manuscript.

REFERENCES


