STUDY ON THE GROWTH OF BACILLUS VELEZENSIS M2 AND APPLYING IT FOR TREATMENT OF THE CATTLE SLAUGHTERHOUSE WASTEWATER

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ABSTRACT

Wastewater from cattle slaughterhouse usually contains high COD and total nitrogen concentration, which required to be treated properly in order to prevent pollution. Strain Bacillus velezensis M2 isolated from Thinh An slaughterhouse, has high ability to degrade organic pollutants rapidly and, therefore, it can be used to produce biological product for slaughterhouse wastewater treatment in large scale, thus reduce setting-up time of operation. In this study, we showed that the best conditions for Bacillus velezensis M2 to grow are NA medium, initial pH of 6.5, inoculum of 3 % (v/v), shaking of 200 rpm. For test in laboratory-scale of 500 ml flask, Bacillus velezensis M2 was induced in the cattle slaughterhouse water containing COD of 1260 mg/L and total nitrogen of 137 mg/L, shaking at 200 rpm, inoculum 3 %, after 12 hours the removal efficiency in COD and total nitrogen were 93.2 % and 83.5 %, respectively.

Keywords: Bacillus velezensis, COD, slaughterhouse wastewater, nitrogen, biomass.

1. INTRODUCTION

Features of the slaughter wastewater are dependent on the type and number of cattle being killed daily. Slaughterhouses wastewater contains a high amount of organic component, grease, and other nitrogen-containing compounds (protein and amino acids), COD: 1000 - 10000 mg/L, BOD₅: 1000 - 8000 mg/L, TN: 100 - 800 mg/L, TP: 20 - 100 mg/L, and fat 20 - 400 mg/L [1 - 5]. Therefore, the control and treatment of slaughter wastewater a heavily polluted effluent, should be particularly concerned for preventing of environmental pollution.

Ammonium (NH₄⁺) composes to main nitrogen compounds in slaughterhouse wastewater and the consequential conversion of these compounds in the nature usually resulted eutrophication effects that killed aquatic beings and resulted re-pollution.

Recently, many different methods are used in slaughter wastewater treatment. For treatment of organic pollutants in wastewater, the most appropriate and effective solution is biological treatment [6]. The essence of biological treatment is that microorganisms metabolize organic
pollutants for their growth and development. Taking advantages of that, we can apply microorganism in order to convert the organic pollutants (environmental contamination) for earning microbial biomass (separable harvest in form of activated sludge). This proposition guides technological solution developments that can handle and exploit resources in polluted wastewater, towards W2E treatment technology (Wastewater to Energy). A new technology is expected to integrates those highlighted features simultaneously: the ability to quickly and strongly convert organic pollutants into biomass; an efficient separating of activated sludge during the biological treatment (to avoid biomass autolysis back into secondary metabolites which not only decrease total biomass but also increase pollutants); and this technology will simultaneously have an competitive advantage in total treatment expense to these current technologies [7].

This study examined several fermentation conditions of Bacillus velezensis M2 in the artificial environment with the purpose of gaining the most biomass. Besides, we tested the slaughter wastewater treatment ability of B. velezensis M2 using aerobic biological methods. Fermentation conditions were optimized for biomass accumulation and for separation of partial activate sludge during treatment, as well as for highest rate of organic pollutants conversion.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Wastewater

Wastewater was collected at slaughterhouses which were in daily operation (early around 4 A.M). Wastewater was taken from the sources in pig slaughter areas, Thinh An, Hanoi. Samples were stored in a refrigerator at 4 °C.

2.1.2. Bacterial strain

B. velezensis M2 was isolated and selected in the laboratory of Department of Microbiology - Biochemistry - Molecular biology, Hanoi University of Science and Technology [8].

2.1.3. Chemicals and equipment

**Chemicals:** K$_2$Cr$_2$O$_7$, H$_2$SO$_4$, Ag$_2$SO$_4$, (NH$_4$)$_2$Fe(SO$_4$)$_2$·6H$_2$O, HgSO$_4$, Ferroin, C$_3$H$_7$O$_3$Na, C$_8$H$_{12}$O$_7$Na$_3$, 2H$_2$O, Fe(CN)$_5$NONa$_2$.2H$_2$O, C$_2$N$_3$O$_5$Cl$_2$Na$_2$.H$_2$O, NaOH, K$_2$S$_2$O$_8$, H$_3$BO$_3$.

**Origin:** Merk, India, China.

**Equipments:** HIRAYAMA pasteurization autoclave (Japan), pH meter, VBEG - ML02 shacker, spectroscopy, microscopy.

2.2. Methods

2.2.1. Collection and storage of wastewater
Wastewater samples were taken at Thinh An - a pig slaughterhouse where raw wastewater concentrate. Samples were stored in 10L container and maintained at 4 °C during transportation and storage in the laboratory.

2.2.2. Effects of cultivation conditions on B. velezensis M2 biomass

Preparation of starter culture: 0.1 ml from seeding culture was propagated in a 500 ml flask containing 100 ml of NA medium at 37 °C, shaking at 200 rpm, for 24 hours. The inoculum obtained from culture in the logarithmic phase was used for the next experiments [9].

Fermentation conditions: 500 mL flasks, 100 mL medium (pasteurized at 121 °C for 20 minutes), at 37 °C, shaking speed of 200 rpm. The above conditions were maintained during the experiments. The effects factors on biomass fermentation, including parameters: pH, inoculum rate and media components, were determined.

2.2.2.1. Effects of pH on B. velezensis M2 biomass

B. velezensis M2 was inoculated at 5% volume of starter culture and cultivated in above mentioned conditions with pH adjusting in the range from 6.0 – 9.0 with a jump of 0.5. After 24 hours of fermentation, biomass was determined by measuring OD at 600 nm.

2.2.2.2. Effects of inoculum rate on B. velezensis M2 biomass

Experiments were controlled on the above conditions with pH 6.5 and different inoculum ratios: 1 %, 3 %, 5 %, 7 %, and 10 %. After 24 hours of fermentation, biomass was determined by measuring at OD_{600 nm}.

2.2.2.3. Effects of culture components

The effects of culture components was determined by applying the NA medium with changing of peptone proportions (0.3 %, 0.5 %, 0.7 %, 1.0 %) (w/v) in rank meat extract (0.09 %, 0.15 %, 0.21 %, 0.3 %) (w/v). Other conditions of the fermentation process were kept constant during the fermentation. After 24 hours of fermentation, biomass was determined by measurement of OD_{600 nm}.

2.2.3. Wastewater treatment methods in laboratory scale

The experiment consists of two 500 ml flasks containing 100 ml of wastewater. One control flasks (no additional B. velezensis M2) and the other flask were inoculated with 3 % (v/v) seeds (seeds obtained after 24 hours of fermentation of B. velezensis M2 and centrifuged at 6000 rpm). After 24 hours, the biomas from 2 flasks were collected by centrifugating at 6000 rpm for 10 minutes. This biomass was then added to 2 flasks as above mentioned (100 ml per 500 ml flask) and flasks of wastewater were incubated at shaking of 200 rpm. The samples were taken at the beginning of process and after that, once per every 2 hours. Samples were centrifuged at 6000 rpm for 10 minutes, removing residues, analyzing COD, NH_{4} - N, pH to monitor wastewater treatment ability of B. velezensis M2. The sample process was terminated when the pollution parameters stopped changing.

2.2.4. Analytical methods
The COD, nitrate, nitrite, ammonia were analyzed according to TCVN [10]. The pH was measured using pH meter. The analyses were conducted at the laboratory of Microbiology – Biochemistry – Molecular Biology at Hanoi University of Science and Technology.

3. RESULTS AND CONCLUSION

3.1. Effects of cultivation conditions on B. velezensis M2 biomass production

3.1.1. Effects of initial pH on B. velezensis M2 biomass production

Ion concentrations H⁺ or OH⁻ alter the separation of substances in the environment, so pH level has multiple effects on microorganism metabolism, growth and development. After 24 h of fermentation, absorbance at 600 nm was measured to determine the biomass of B. velezensis M2 and shown in Fig. 1.

The results showed that biomass of B. velezensis M2 intensive increasing in the pH range from 6 - 7.5 than in the alkaline pH range. And, at pH 6.5 biomass concentration was the highest after 24 hours of fermentation (OD\textsubscript{600 nm}: 3.95). In conclusion, B. velezensis M2 developments the best at pH neutral to slightly acidic. It is in agreement with Xiangyang Liu et al. [11] where B. velezensis H3 was isolated and examined, they also showed that largest biomass collection was obtained at pH 6. Thus, pH is confirmed as an important factor to grow biomass of B. velezensis M2

Figure 1. pH effects biomass of B. velezensis M2.

3.1.2. Effects of inoculum rate

The determination of biomass of B. velezensis M2 was carried out by measuring absorbance at 600 nm after 24 h of fermentation and the obtained result was shown in Fig. 2.

In different inoculum ration, the OD\textsubscript{600 nm} values were significant differences. The result showed that highest OD\textsubscript{600 nm} obtained was 4.207 with inoculum rate of 3% inoculum ratio of 3 - 7%, biomass concentration obtained was higher than at the other ratio. At 1%, biomass concentration was the lowest (with OD\textsubscript{600 nm}: 2.68). Therefore, the suitable ratio was selected at 3% for the next experiment.

Figure 2. Inoculum rate effects biomass of B. velezensis M2.
3.1.3. Effects of culture components

After 24 hours of fermentation, absorbance at 600 nm was measured to determine the biomass. Result was shown in Fig. 3.

![Figure 3. Culture components effects of B. velezensis’s biomass M2. 1: 0.3 % peptone; 0.09 % meat extract, 2: 0.5 % peptone; 0.15% meat extract, 3: 0.7 % peptone; 0.21 % meat extract, 4: 1 % peptone; 0.3 % meat extract.](image)

B. velezensis M2 was able to grow and develop in all four media. According to this result, B. velezensis M2 grew best in the fourth medium, the biomass concentration at OD$_{600}$ nm: 4.27. At first medium, the strain grew slower and had low biomass concentration (with OD$_{600}$ nm: 1.307). While at the second and the third media, this strain grow intensively, however less than the fourth media. In conclusion, B. velezensis M2 grew and developed best in medium contained 1 % peptone and 0.3 % meat extract.

3.2. The ability of the slaughterhouse water waste treatment of B. velezensis M2

3.2.1. Characteristics of influent wastewater

Wastewater was collected at the slaughterhouse where the effluent contained mostly of pig’s blood. The result of wastewater analysis is illustrated in Table 1. The proportion of BOD$_5$ to COD is 0.83, which is compatible for using biological treatment and the biomass-harvesting model in this project.

From technological of view, the higher amounts of protein will be treated or applied for secondary reuse purposes (for example to harvesting more biomass), it leads to face a strong environmental NO$_3^-$ polluting.

<table>
<thead>
<tr>
<th>pH</th>
<th>TSS (mg/L)</th>
<th>Protein (mg/L)</th>
<th>BOD$_5$ (mg/L)</th>
<th>COD (mg/L)</th>
<th>TP (mg/L)</th>
<th>TN (mg/L)</th>
<th>N-NH$_4^+$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>50</td>
<td>980</td>
<td>1050</td>
<td>1260</td>
<td>10</td>
<td>137</td>
<td>15</td>
</tr>
</tbody>
</table>

3.2.2. The ability of slaughterhouse wastewater treatment of B. velezensis M2

The result of the COD and TN treatment of B. velezensis M2 after 24 hours is illustrated in Figure 4 and 5.
After 12 hours, the highest reduction of COD and TN by added strain were observed at 93.2 % (86 mg/L), 83.5 % (31 mg/L) and reduction in control sample were 48.4 % (650 mg/L) and 24.8 %, correspondingly. Thus, the applying of \textit{B. velezensis} M2 resulted in a positive effect for the wastewater treatment. Nevertheless, during the 24-hour treatment process, COD and TN in control sample continued to reduce to 61.9 % (480 mg/L) and 30.7 %, correspondingly, whereas, in treated sample, COD and TN slowly increased (158 mg/L and 33 mg/L).

This phenomenon could be explained as the starvation of microorganisms when substrates in the environment were exhausted, which led to cell death and autolysis, in turns, contaminating the environment with secondary metabolites. It demonstrates that biomass separation process.

**4. CONCLUSION**

Optimal conditions for \textit{B. velezensis} M2 to produce produces biomass is NA culture media including: 1 % peptone, 0.3 % beef extract, 0.5 % NaCl, pH 6.5, volume ratio is 3 % (v/v).

Slaughterhouse wastewater is polluted with high amounts of organic compounds. The applying of \textit{B. velezensis} M2 for treating this wastewater has resulted in positive effects on COD and TN reduction, observed at 93.2 %, 83.5 %, correspondingly, after 12 hours of the treatment.

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TÓM TÁT

KHẢO SÁT CÁC ĐIỀU KIỆN NUÔI CHỪNG B. VELEZENSIS M2 VÀ THỦ NGHIỆM NĂNG LỰC XỬ LÍ NƯỚC THẢI GIẾT MÔ GIA SỨC

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Nước thải của các lò giết mổ gia súc thường có hàm lượng hữu cơ cao nếu không được xử lý sẽ gây ô nhiễm nghiêm trọng đến môi trường. Chủng *B. velezensis* M2 được phân lập từ nguồn nước thải giết mổ huyện Thịnh An có năng lực xử lý hợp chất hữu cơ cao trong thời gian ngắn nên được ứng dụng trong nghiên cứu tạo chế phẩm để tiết giảm thời gian khởi động hệ thống xử lý nước thải giết mổ quy mô lớn. Kết quả khảo sát ảnh hưởng các điều kiện nuôi chăn của *B. velezensis* M2 thu sinh khối cho thấy điều kiện tốt nhất là sử dụng môi trường NA, pH ban đầu 6,5, tỉ lệ cấp giống 3 % (v/v), nuôi lắc 200 vòng/phút. Thử nghiệm năng lực xử lý nước thải với COD và tổng nitrơ đầu vào là 1260 mg/L, 137 mg/L của chủng *B. velezensis* M2 trên quy mô bình tam giác 500 ml nuôi lắc 200 vòng/phút với tỉ lệ cấp giống 3 % sau 12 giờ hiệu suất xử lý COD và tổng nitrơ tương ứng là 93,2 % và 83,5 %.

*Từ khóa:* Bacillus velezensis M2, COD, nước thải giết mổ gia súc, nitrơ, sinh khối.