Isolasi dan seleksi mikroba pendegredasi asetonil serta karakterisasi enzim yang berperan di dalamnya

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Abstrak

Acetonitrile is an organic, derivative of carboxylic acid, and toxic compound. This compound has been widely used in pharmaceutical and chemical industries. Nowadays, there are more interests in acetonitrile-degrading microbes for their potential in chemical syntheses and biological detoxification of nitrile-containing wastes.

The aim of this study were to isolate, select, and characterise the isolate from industrial effluents which has the best degrading capability and its acetonitrile-degrading enzyme. Cultures were grown on mineral medium with microelements and acetonitrile was added as sole source of energy, carbon, and nitrogen. Analysis to characterise the acetonitrile-degrading enzyme had been conducted with whole cells of the selected isolate. Decreasing of acetonitrile concentration and formation of its degrading products were determined by gas chromatography and ammonia analysis was done by Nessler's method.

Isolate D5, identified as Corynebacterium sp., was able to grow on high concentration acetonitrile (up to 5 % v/v) and exhibited the highest specific growth rate (p) among 29 isolates which could grow on acetonitrile. When Corynebacterium D5 grew on 2 % (vlv) acetonitrile, the doubling time was 6 hours 40 minutes, the specific growth rate (p.) was 0.1 h-1, and the acetonitrile decreasing rate was 3.99 mM/h. Increasing of acetonitrile concentration would extend the doubling time, decline the maximum growth and specific growth rate (M), and biomass production. The products of acetonitrile degradation by Corynebacterium D5 were acetamide, acetic acid, and ammonia. The highest maximum growth of Corynebacterium D5 showed when 13-aminopropionitrile was used as a substrate.

Corynebacterium D5 degraded 5 % (v/v) acetonitrile with degrading rate of 0.906 µmol min-' (mg dry weight cells)-'. Corynebacterium D5 hydrolysed acetonitrile by two-step reaction catalysed by nitrile hydratase and amidase. The acetamide forming rate [0.399 pmol min' (mg dry weight cells)-'] was higher than acetic acid forming rate [0.198 µcool min-' (mg dry weight cells)"] and the maximum acetamide concentration formed (about 239 mM) was also higher than maximum acetic acid concentration formed (about 145 mM). Nitrite hydratase activity of Corynebacterium D5 was found to be higher than amidase activity. Maximum nitrite hydratase activity was found out at pH 6 and at 30 °C, while maximum amidase activity was found out at pH 7 and up to 60 °C the activity still increase. Nitrite hydratase of Corynebacterium D5 was totally inhibited by 5 mM Hg2+, whereas amidase was slightly inhibited by 10 mM Co2+.