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Sewage Treatment Plant Sludge: A Source of Potential Microorganism for Citric Acid Production

Parveen Jamal, Md. Zahangir Alam, M. Ramlan, M. Salleh and Munirah M. Akib
Bioenvironmental Engineering Research Unit (BERU),
Department of Biotechnology, Engineering Faculty of Engineering,
International Islamic University Malaysia (IIUM) Jalan Gombak,
53100 Kuala Lumpur, Malaysia

Abstract: This research emphasizes on the utilization of Sewage Treatment Plant (STP) sludge, which is an inexpensive and easily available raw material and a good source for growth of microorganisms because it has enough nutrients and trace elements. This can be considered as an alternative cost effective solution for waste management in addition to the production of a value added product citric acid, one of the important chemicals used in various industrial processes. The isolation of filamentous fungi especially *Aspergillus* was made from STP sludge for better adaptability. Six strains of *Aspergillus* were isolated from STP sludge and identified using slide culture technique followed by image analysis. Four strains (SC906, A103, A2017 and A1020) were selected from lab stock. All strains were screened under controlled fermentation conditions such as pH range of 2-3, temperature 30°C and agitation 150 rpm, using 1% (w/w) of substrate (STP sludge), 2% (w/w) co-substrate (wheat flour) with inoculum's size of 2% (spore mL^{-1}), using a liquid state fermentation process for the maximum production of citric acid. Evaluation of fungal potentiality was done in terms of maximum citric acid production, biosolids production (TSS %) and Chemical Oxygen Demand (COD) removal. Strain A-SS106 produced the highest concentration of citric acid (0.14 g L^{-1}), TSS (15.18 g L^{-1}) and COD removal (90.1%) on the fourth day of fermentation.

Key words: Isolation, screening, fermentation, COD removal and liquid state bioconversion

INTRODUCTION

STP sludge is one of the final products for the treatment of sewage at a sewage (wastewater) treatment plant. Treatment breaks down the organic matter and kills disease-causing organisms[1]. The main groups of the organic solids in the sludge are protein, carbohydrates, fats and oils[2], which vary with their origin, system and efficiency of the wastewater treatment plant[3]. In Malaysia, STP sludge is the largest contributor of organic pollution of water resources and the environment. Its contribution is top listed (64.4%), followed by animal husbandry wastes (32.2%), agro-based (1.7%) and industrial effluent (1.3%) in terms of BOD load[4]. In Malaysia, approximately 3.8 million cubic meters of STP sludge are produced by Indah Water Konsortium (IWK) annually and the total cost of managing was estimated as RM 1 billion[5]. This sludge volume is expected to rise to 7 million cubic meters by the year 2020. The management of the ever increasing organic wastes has been one of the important environmental issues in Malaysia, which requires a pragmatic and economic approach and study to utilize this sludge is vital to have a good waste management. STP sludge can be a very good source of carbon, nitrogen, phosphorus and other nutrients for many microbial processes that can add to the value of sludge by producing valuable metabolic products like citric acid.

Citric acid is one of the important chemicals used in various industrial processes. It is estimated that about 500,000 tons of citric acid are produced annually by fermentation of expensive raw materials like glucose and sucrose. In developed countries, 65% of citric acid consumption is in food and beverages and 20% in household detergents with the estimated global growth rate in demand between a 4 to 5% per year[6]. Various substrates like sugar cane molasses, inulin, kurma, date fruit syrup and carob pod[7-11] have been used for citric acid production by *Aspergillus Niger*. Some products, which were produced by using the microorganism *A. Niger*, have been assessed as acceptable for daily intake by the World Health Organization[12]. Therefore, this study emphasized on utilization of a new substrate, sewage treatment plant sludge (STP sludge) for the production of citric acid as well as removal of COD. In order to achieve the target, isolates of filamentous fungi especially *Aspergillus* was done from STP sludge itself

Corresponding Author: Parveen Jamal, Bioenvironmental Engineering Research Unit (BERU), Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia (IIUM) Jalan Gombak, 53100 Kuala Lumpur, Malaysia, Phone: +603-20564558, Fax: +603-20564442

for better adaptability and its screening for effective bioconversion of the sludge into citric acid.

MATERIALS AND METHODS

Sample collection: STP sludge sample was collected from Indah Water Konsortium (IWK) Sewage Treatment Plant, Damansara, Kuala Lumpur, Malaysia. The sample was stored at 4°C for further use as a substrate. Wheat flour is used as a co-substrate procured from the market.

Isolation, purification and identification of microorganisms: The media used for isolation of microorganism from the sample (STP sludge) was Rose Bengal Agar (RBA). The media composition was a modified composition used by Ilias[13] (per liter); KH_2PO_4 1.0, $MgSO_4.7H_2O$ 0.5, Peptone 5.0, Dextrose 10.0, Rose Bengal Agar 0.35, Agar 10.0 and Streptomycin 2 mL (50 mg mL^{-1}). All compositions were added prior to autoclaving at 121°C for 20 minutes, except Streptomycin, which was sterilized and added to the media after autoclaving. Dilution was done by mixing 5ml of sample with 100ml sterile distilled water. Afterwards, 1 ml of diluted sample was put into a Petri dish followed by 20ml of RBA media (3 replicates) and allowed to grow for 3-4 days in incubator at 32°C. Fungi were sub-cultured on Potato Dextrose Agar (PDA) medium to obtain pure strains. Identification was done visually and by micro-morphological studies using the Slide Culture Technique[17]. Image Analysis System (IAS), consisting a microscope, a CCD camera, a PC and image analysis software (Olympus Micro image Lite 4.0) was used to determine the morphology of the isolates.

Inoculum preparation: Inoculum preparation (spore suspension) was done according to the method suggested by Alam *et al*.[14]. Cultures grown on PDA medium in Petri dishes at 32°C for 7 days were transferred into an Erlenmeyer flask (250 ml) containing 100 ml of sterile distilled water. It was shaken in a rotary shaker for 24 hours with 150 rpm and filtered. The filtrate was used as inoculum after measuring its concentration (spores mL^{-1}) by Haemocytometer.

Screening: Screening was done to get the best strain based on maximum citric acid production, treated biosolids and COD removal. Ten strains of *Aspergillus* were selected for screening based on assessment of the best adapted strains in STP sludge. Six of them (A-SS101, A-SS102, A-SS104, A-SS105, A-SS106 and A-SS107) were selected from isolated strains and another four (A103, A1020, A2017 and SC906) from the lab stock. The screening experiment was done in a 500 ml of the Erlenmeyer flask containing 100 ml of wastewater sludge with the fixed process conditions according to the literature: substrate concentration of 1% (w/v), co-substrate concentration 2% (w/v), initial pH of 3, temperature of 30°C, agitation of 150 RPM and inoculum size of 2% (w/v). The sample was sterilized, inoculated and incubated in a rotary shaker for 2, 4 and 6 days of treatment. After treatment the sample was harvested to determine the parameters citric acid, chemical oxygen demand (COD) and biosolids content. Citric acid was determined according to the method of Marier and Boulet[15]. COD and biosolids as the total suspended solids (TSS) were measured following the methods of APHA[16].

RESULTS AND DISCUSSION

Six strains of filamentous fungi - A-SS101, A-SS102, A-SS104, A-SS105, A-SS106 and A-SS107 (Fig. 1)) - were isolated from STP sludge and identified tentatively as *Aspergillus* by micro-morphological studies using slide culture technique[17] and by examining the size, shape arrangement and development of conidiophores and phialospores with IAS (Fig. 2).

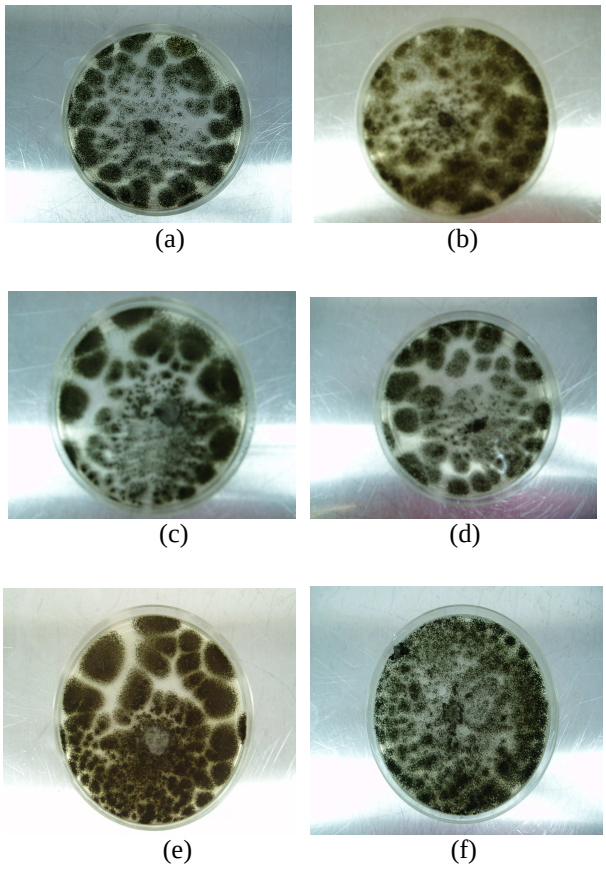

Fig. 1: Six strains of *A. Niger* isolated from STP sludge growing on PDA at 32°C; (a) A-SS101, (b) A-SS102, (c) A-SS104, (d) A-SS105, (e) A-SS106 and (f) A-SS107

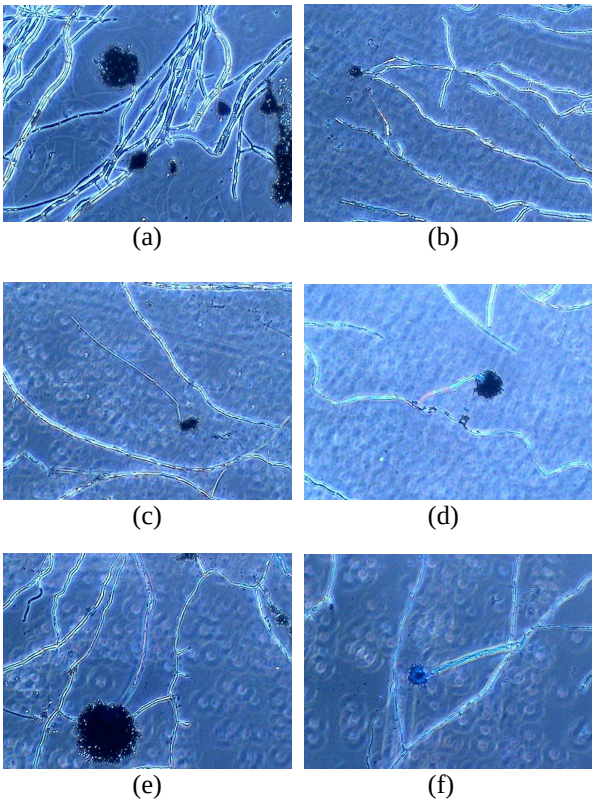

Fig. 2: Reproductive structures of the strains isolated from STP sludge (a) A-SS101, (b) A-SS102, (c) A-SS103, (d) A-SS104, (e) A-SS105 and (f) A-SS106

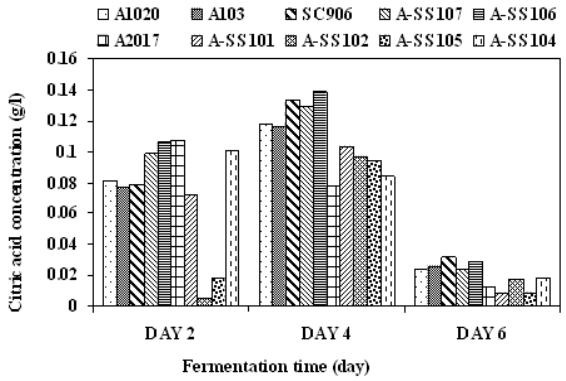

Fig. 3: Citric Acid concentration varies with fermentation time

Four strains of *Aspergillus* (SC906, A103, A2017 and A1020) were selected from lab stock. All ten strains were screened using same process conditions and the best strain (A-SS106) was selected on the basis with citric acid produced, COD removal, and treated biosolids. Citric acid concentration varies with fermentation time as shown in Fig. 3. High yield of citric acid was produced by all strains on 4th day of fermentation except for A2017 and A-SS104 and it reduced when the fermentation time increased.

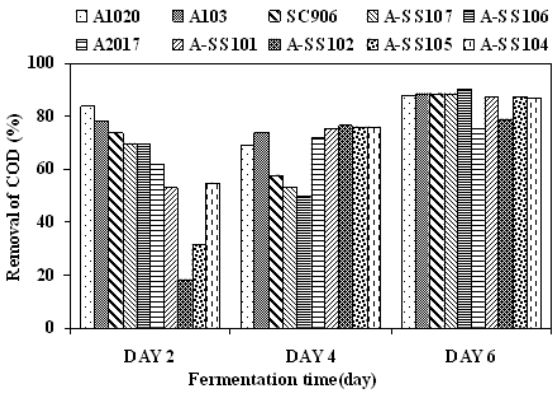

Fig. 4: Percentage removal of chemical oxygen demand (COD) varies with fermentation time

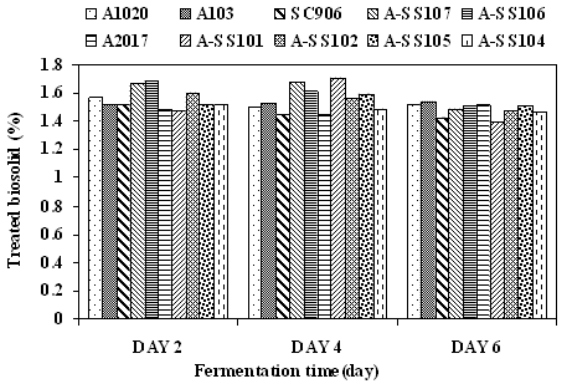

Fig. 5: Percentage of treated biosolids varies with fermentation time

Although these two strains can produce highest citric acid in less time, the yield is insignificant (0.017 g L^{-1} and 0.101 g L^{-1} for A2017 and A-SS104 respectively) compared to other strains. Overall, the highest citric acid produced (0.139 g L^{-1}) was by strain A-SS106 on 4th day and the lowest (0.005 g L^{-1}) was by A-SS105 on the 2nd day of fermentation. The difference of maximum citric acid yield for SC906 and A-SS106 is 0.006 g L^{-1} for day 4 and 0.027 g L^{-1} for day 2. For day 6, citric acid production of SC906 is higher than A-SS106 by only 0.03 g L^{-1}. Since A-SS106 obtained higher citric acid compared to SC906 for most of days, strain A-SS106 was selected for further study. The amount of citric acid produced during this study is very little, presumably due to the presence of heavy metals and other components in STP sludge[14], which can decrease the production of this acid.

The second parameter used to evaluate the fungal potential was COD. Removal of COD increased with fermentation time as shown in Fig. 4. This observation was expected as removal of COD is a percentage of the organic matter removed during the treatment because it is consumed by the fungi. The maximum COD removal

(90%) was observed on day 6 for most of the strain. Since there is not much difference in terms of COD removal for all strains in terms of maximum removal and time of fermentations, A-SS106 can be selected as the potential strain for maximum COD (90.1%) removal. When we observed the percentage of treated biosolids (Fig 5), strain A-SS106 showed a good growth of biomass (1.574%) on day 2 and decreased as the time of fermentation increased. Other strains namely A103, A-SS107, A-SS101 and A-SS105 reached their optimum values (0.5238, 1.6778, 1.71 and 1.5942% respectively) on the 4th day of fermentation. Since none of the strains have a significant difference in percentage of treated biosolids when compared to each other, A-SS106 could be considered the best because it reached its maximum in day 2.

CONCLUSION

By evaluating the fungal potentiality in terms of citric acid concentration, COD removal and treated biosolid percentage, we reached to a conclusion that the best strain is A-SS106. This strain was selected for further optimization studies using STP sludge as substrate (results not included in this paper) because, besides producing the highest citric acid concentration, it also achieved optimally treated biosolid percentage and removal of COD in a short time compared to other strains.

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