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ORIGINAL ARTICLE

Use of wood vinegar to enhance 5-aminolevulinic acid production by selected *Rhodopseudomonas palustris* in rubber sheet wastewater for agricultural use

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Abstract This study aimed to produce inexpensive 5-aminolevulinic acid (ALA) in a non-sterile latex rubber sheet wastewater (RSW) by *Rhodopseudomonas palustris* TN114 and PP803 for the possibility to use in agricultural purposes by investigating the optimum conditions, and applying of wood vinegar (WV) as an economical source of levulinic acid to enhance ALA content. The Box–Behnken Design experiment was conducted under microaerobic-light conditions for 96 h with TN114, PP803 and their mixed culture (1:1) by varying initial pH, inoculum size (% v/v) and initial chemical oxygen demand (COD, mg/L). Results showed that the optimal condition (pH, % inoculum size, COD) of each set to produce extracellular ALA was found at 7.50, 6.00, 2000 for TN114; 7.50, 7.00, 3000 for PP803; and 7.50, 6.00, 4000 for a mixed culture; and each set achieved COD reduction as high as 63%, 71% and 75%, respectively. Addition of the optimal concentration of WV at mid log phase at 0.63% for TN114, and 1.25% for PP803 and the mixed culture significantly increased the ALA content by 3.7–4.2 times (128, 90 and 131 μM, respectively) compared to their controls. ALA production cost could be reduced approximately 31 times with WV on the basis

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of the amount of levulinic acid used. Effluent containing ALA for using in agriculture could be achieved by treating the RSW with the selected ALA producer *R. palustris* strains under the optimized condition with a little WV additive.

1. Introduction

In agriculture, 5-aminolevulinic acid (ALA) can be an effective and non-toxic biodegradable herbicide and insecticide (Sasaki et al., 2002) including an ability to regulate several key physiological processes associated with plant growth under saline conditions through improvements to the antioxidative defense systems and photosynthetic electron transport under stress conditions (Nunkaew et al., 2014). ALA is known as an essential biosynthetic precursor for all heterocyclic tetrapyrroles such as chlorophyll, vitamin B_{12}, and other specialized compounds in higher plants, algae, and bacteria (Akram and Ashraf, 2013). In phototrophic bacteria, it is synthesized via the Shemin pathway with two precursors, succinate and glycine, brought together by the enzyme aminolevulinic synthase (ALAS) (Liu et al., 2005). ALA itself is used for the synthesis of porphobilinogen (PBG) via the action of aminolevulinic dehydratase (ALAD) (Heinemann et al., 2008); thereby levulinic acid as an inhibitor of ALAD has been used to increase ALA production.

It is also well recognized that ALA at low concentrations can promote the growth of many plants including rice (Hotta et al., 1997; Nunkaew et al., 2014). Unfortunately, commercial ALA is too expensive for use in agricultural applications. Hence, the production of ALA by microorganisms has been suggested as an attractive alternative source because microbes can use a variety of raw materials to produce ALA with a high productivity but a low pollution (Kamiyama et al., 2000; Kang et al., 2012). Among the microbes that produce ALA, purple nonsulfur bacteria (PNSB), are good candidates for ALA production as they are versatile organisms that grow phototrophically and/or heterotrophically in various wastewaters under anaerobic–microaerobic/light conditions and aerobic dark conditions, respectively (Kantachote et al., 2005; Kars and Ceylan, 2013). They are often found in the wastewater that is being treated in open air lagoons (Kornochalert et al., 2014a,b). Selected PNSB strains have been extensively used to study ALA production by optimizing the amounts of succinate, glycine and volatile fatty acids (Saikeur et al., 2009; Sattayasamitsathit and Prasertsan, 2014). Nevertheless, those compounds are expensive to use; and thus perhaps we should try to use a much cheaper alternative substrate such as wastewater to reduce the costs of ALA production and to facilitate its use in agriculture.

Thailand has become one of the world's major producers of natural rubber and rubber plantations now exist in most parts of the country; consequently there has been a huge increase in the numbers of cooperative smoked rubber sheet factories (CSRFs) throughout Thailand. Wastewater from CSRFs is normally treated by anaerobic lagoons as they are of low cost and easy to operate (Chaiprapat and Sdoodee, 2007; Kornochalert et al., 2014a,b). However, this can produce serious problems of rotten egg odors (H_2S) thus more appropriate technologies have been developed to use PNSB such as *Rhodopseudomonas blastica* DK 6 and *Rhodopseudomonas palustris* P1 to remove 90% and 98% COD in non sterile latex rubber sheet wastewater (RSW), respectively without the production of H_2S (Kantachote et al., 2005; Kornochalert et al., 2014b). At the same time, the shortage of water for agricultural use is due to the increasing urbanization and industrialization; therefore, the concept to reuse wastewater after treatment could lead to solve this problem. Fortunately, our previous work with the use of 3% *R. palustris* P1 could treat RSW to produce effluent that met Thai standard guidelines, and the effluent could directly be used as irrigation water (Kantachote et al., 2010).

Hence, it should be possible to produce inexpensive ALA in RSW by concomitant with treating RSW. However, to increase the amount of ALA production by PNSB, levulinic acid has been used as a competitive ALAD inhibitor as previously mentioned (Saikeur et al., 2009; Kars and Ceylan, 2013). Unfortunately, this increases the cost of ALA production. Wood vinegar (WV) is a byproduct from charcoal production and contains 12–17 mM levulinic acid (Matsushita et al., 2002). Hence, it would be attractive to try to use WV as a levulinic acid source for producing ALA by PNSB with an economical cost, and our work described here is the first known to investigate this possibility. According to the above information, the objectives of this study were to investigate the optimal conditions for ALA production in RSW by our ALA producer *R. palustris* strains (Nunkaew et al., 2015) and the use of WV containing levulinic acid to enhance the ALA content. This might lead to the possibility to use RSW effluent containing ALA for agricultural purposes like irrigation water to ameliorate rice growth in saline soil.

2. Materials and methods

2.1. Preparation of latex rubber sheet wastewater

Latex rubber sheet wastewater was collected from a lagoon pond of a CRSF at the Phichit suburb in Songkhla Province, Thailand. The wastewater was collected as a composite sample at a deep level roughly 20–100 cm from the surface at various positions of the pond for obtaining a representative sample and pouring them into a 25 L plastic tank. The collected wastewater was filtered using a cheesecloth to remove solid particles, and directly used as the raw wastewater medium without supplementing with any nutrients, and with no autoclaving (RSW).

2.2. ALA producing PNSB inocula

The selected *R. palustris* strains (TN114 and PP803) from our previous study (Nunkaew et al., 2015) were used for this study

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