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RESEARCH

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Effective utilization of wastewater for valuable validamycin A biosynthesis by *Streptomyces hygroscopicus* K2509 in plant-scale bioreactor

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Abstract

Background: The continuous escalation in wastewater production with declining dependency on conventional resources as a result of rapid urbanization, increasing global water scarcity and growing population have initiated many researchers to look out for efficient means of utilizing wastewater. In this regard, an effectual process economizing approach has been achieved, upon utilizing the discharged wastewater from validamycin A recovery process in fermentation medium as a replacement of costly tap water.

Results: In this study, wastewater was successfully used as a fermentation medium for the production of validamycin A in plant-scale bioreactor. Moreover, a new strain *Streptomyces hygroscopicus* K2509 was screened showing a good production capability in this low-cost culture environment and showed maximum validamycin A production and productivity of 21.23 g/L and 0.29 g/L h, respectively. Execution of this study has managed the effective utilization of wastewater by reducing 12.42% production cost per kilogram of validamycin A in an environmental friendly way.

Conclusion: The novel successful approach for using process wastewater even on being executed in plant-scale bioreactor was proved cost effective. In short, the presented effective way of utilizing wastewater in study will definitely serve as potentially cheap fermentation medium upon replacing tremendously used expensive tap water.

Keywords: Fermentation, Wastewater, Validamycin A, Plant-scale bioreactor, Cost effective

Background

Validamycin A, a kind of biofungicide, is extensively used to treat rice blast, false smut, and sheath blight (Horii et al. 1971). The chemical structure of validamycin A being similar to the active fraction of trehalase facilitates validamycin A binding with the trehalose to inhibit its enzymatic hydrolysis (Müller et al. 1995). Thus, validamycin A could inhibit the fungus which consumes the trehalose as energy-storage material, at the same time, doing no harm to mammal, fish, and poultry (Asano et al. 1987). Even after considering the advantage of inhibiting fungus growth, the price of validamycin A still remains higher than some other agricultural chemicals such as tebuconazole and hexaconzole. Therefore, there is a need to find a more cost-effective fermentation medium for validamycin A production that might reduce the current cost of production and further improve its market acceptability.

Culture medium, energy, vapor, and tap water are considered as the significant cost factors for fermentation processes that play an important role in total production cost. In order to economize the fermentation process, many kinds of residue or waste material in industry were employed as alterative medium component, such as corn fiber (Chen et al. 2011), orange peels (Mohsin

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et al. 2018), straw (Meena et al. 2015), sugarcane bagasse hemicellulose (Tsigie et al. 2011), and cane molasses (Liu et al. 2008). These were successfully used for the production of bulk chemicals. However, little attention has been focused on the utilization of the industrial wastewater for bioprocesses. The main reason behind this might be the presence of complicated or toxic components in the wastewater, which can cause harmful impacts on physiological characteristics of microorganisms during fermentation process (Sa'Idi 2010).

On the other hand, reutilization of water can help to close the loop between water supply and wastewater disposal (Asano 1998). Reusing water includes two fundamental functions: (1) the treated effluent is used as a water resource for beneficial purposes; (2) the effluent can be kept out from the streams, lakes, and beaches, thus reducing the pollutions of surface water and groundwater (Asano et al. 1992). By implementing this method, the integrated water resources management can be more efficient (Saravanan et al. 2010), and in addition, it offers a systematic progress toward safe and reliable water supply practices (Asano 2002).

The recovery process of validamycin A includes two main steps: (1) microfiltration to separate the broth from mycelium, (2) ion-exchange chromatography for the extraction of validamycin A from other soluble materials in broth (Fredenhagen 2004). In general, ion-exchange chromatography method is widely adopted in antibiotic industry as a product-recovering technology for effective removal of the impurities from broth (Roemling et al. 2008). However, it should be noticed that this technique requires a large amount of acid or base water for ion-exchange resin washing, and consequently producing enormous wastewater. For example, in validamycin A recovery process, the total volume of wastewater is about eight times of fermentation broth, which would cause an obvious menace to subsequent environment. This effluent was not included in the organic solvent, so it could be treated by anaerobic digestion directly to reduce the biochemical oxygen demand (BOD), chemical oxygen demand (COD), and ion concentration. After meeting the industrial emission standards, it can be discharged. However, building and running the water-treatment equipment in manufacturing factory would raise the total cost of industry. Therefore, utilizing wastewater is the optimal approach to economize the process in an environmental friendly way.

In this study, we have successfully adopted the ion-exchange wastewater as a substitute of tap water during culture medium preparation for economical production of validamycin A. To the best of our knowledge this process-economizing approach is being implemented for the very first time by the present authors. In this study, fermentation was commenced in 50-L laboratory-scale bioreactors as well as in 60 m^3 plant-scale bioreactors to check its feasibility at larger-scale production. Moreover, a new strain of *Streptomyces hygroscopicus* KN2509 with good validamycin A production and robotic performance in ion-exchange wastewater was screened to be applied in desired fermentation task. The main objective of the study remains on the cost-effective utilization of water resources during production of validamycin A in an environment-friendly manner.

Methods

Microorganism

The strains used in this study were *S. hygroscopicus* K18 and *S. hygroscopicus* K2509, which were preserved by Wuhan Kernel Bio-tech Co., Ltd., P. R. China. The *S. hygroscopicus* K2509 was mutated and screened from the *S. hygroscopicus* K18. Spores of *S. hygroscopicus* were preserved in 20% glycerol at −70 °C.

Preculture and fermentation in the stirred-tank bioreactor

The strain was grown on an agar slant containing 2% (w/v) soybean meal, 4% maltosstudy 5% agar. Mycelium was collected and suspended in normal saline after cultivation for 5 days.

For lab-scale experiments, the preculture medium consisted of the following components (g/L): corn powder 30, soybean powder 22, yeast extract 5, NaCl 2, KH_2PO_4 0.8. Culture pH was adjusted to 7.1–7.5. Eight liter medium was prepared in a 15-L bioreactor, then mycelium from slant culture was inoculated, and after that incubated at 37 °C with agitation speed at 300 rpm and aeration rate of 1 vvm. Fermentation medium (g/L) is listed as follows: corn powder 90, soybean powder 40, yeast extract 10, NaCl 1, KH_2PO_4 1. The 50-L stirred-tank bioreactor (NC-BIO-50L, Shanghai Guoqiang Bioengineering Equipment Co., Ltd., China) with 30 L working volume was used. Fermentation was conducted at 37 °C and inoculated with 10% (v/v) of the precultured inoculum. The agitation and aeration were set at 350 rpm and 1.2 vvm, respectively. The pH was adjusted to 7.5 with continuous addition of 28% (w/w) ammonia solution.

For plant-scale experiments, a 60 m^3 stirred-tank bioreactor was used located at Wuhan Kernel Bio-tech Co., Ltd., China. The culture medium was the same as 50-L bioreactor. The initial working volume was controlled at 44 T and the stirring speed was 150 rpm. Other culture conditions were referred same with the lab-scale experiments.

Analytical methods for parameters

Oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER) were detected by a process mass spectrometer (MAX300-LG, Extrel, NY, USA). Online biomass was measured by Biomass monitor (Aber Instruments Ltd., Aberystwyth, UK). In this study, the selected frequency was 580 kHz, and the low pass was 10, so the correct and flat capacitance curve could be observed.

A total of 50 mL broth was taken from bioreactor rafter at every 6 h for conducting the analyses of reducing sugar concentration and validamycin A concentration.

Glucose concentration was measured by standard phenol-sulfuric acid method (Masuko et al. 2005). Validamycin A production was quantified by HPLC using an Agilent 1200 series (USA) with Hypersil ODS2 column (Wei et al. 2011). All the experiments were performed in triplicate to ensure the reproducibility.

Results and discussion

Validamycin A fermentation by *S. hygroscopicus* K18 and K2509 using tap water

Streptomyces hygroscopicus is extensively applied as validamycin A fermentation microorganism (Iwasa et al. 1970). In our previous study, a new strain: *S hygroscopicus* K2509 was mutagenized from the *S. hygroscopicus* K18, which was preserved by Wuhan Kernel Bio Tech Co., Limited. The physiological and metabolic discrepancies of these two strains cultured with tap water are shown in Fig. 1. The validamycin A titers of K2509 and K18 were 19.04 g/L and 22.01 g/L, respectively (Fig. 1a). The productivity of K18 in the first 42 h could reach 0.43 g/L h, which was 30.0% higher than that of K2509. Figure 1b shows the time course of capacitance, which could be used to characterize the biomass in the broth (Li et al. 2014). This profile illustrated that the maximum capacitance of K18 was 8.3 pF/cm, corresponding to 7.7 pF/cm for K2509. Moreover, the evolutions of DO, reducing sugar shown in Fig. 1c, d further indicated that the metabolic capacity of K2509 was slightly weaker than K18.

Zheng's study (Zheng et al. 2012) demonstrated the primary and secondary metabolism profiles of *S. hygroscopicus* for validamycin A fermentation. The biosynthesis precursors of validamycin A in primary metabolic pathway are glucose-6-phosphate and sedoheptulose-7-phosphate, and glutamine, which are closely related with the biomass-accumulation and substrate-utilization abilities. There was a competitive relationship between the EMP pathway and validamycin A synthesis pathway both of which are rooted from the PP pathway (Zheng et al. 2013). Therefore, the high substrate-usage ability and biomass accumulation might have negative effects on the validamycin A accumulation.

In this experiment, K2509 exhibited a higher final titer but lower productivity compared with K18. This could be ascribed to the lower efficiency of EMP pathway for K2509, which led to lower biomass accumulation and substrate-consumption ability, and consequently resulted in the decreased average productivity. On the other hand, a relatively weak EMP activity would facilitate the carbon flux shift to PP pathway, promoting subsequent validamycin A production.

Validamycin A fermentation by *S. hygroscopicus* K18 and K2509 using ion-exchange wastewater

Ion-exchange method is broadly used in industries as antibiotic recovery strategy. Nevertheless, it results in a production of huge amount of wastewater, which contains inorganic salts, pigments, secondary metabolites, and some unknown intermediate compounds (Yang et al. 2017). All of the components mentioned above are produced during the fermentation process, washed down from the ion-exchange resin, or from other resources. These are generally considered to have unknown effects on microorganism's metabolism. However, the concentration of soluble substances are usually not very high (as shown in Table 1), which makes it possible for some strains with good tolerance ability to grow in it.

In this experiment, two validamycin A-producing strains were cultured in the media with effluent water being discharged from ion-exchange column of validamycin A recovery process. The results are shown in Fig. 2. The final titer of validamycin A of K18 and K2509 were 16.47 g/L and 19.44 g/L, respectively, which were 15.60% and 13.22% lower than the yields obtained using the tap water. This result indicated that the ion-exchange wastewater could inhibit the validamycin A biosynthesis to some extent. Notably, the inhibition effect on K18 was more severe than that of K2509 using ion-exchange wastewater, implying that the K2509 is more robust to withstand harsh environment with ion-exchange wastewater. Furthermore, it should be pointed out that the validamycin A production of K2509 with ion-exchange wastewater was still 0.40 g/L higher than that of K18 with tap water, demonstrating an outstanding fermentation potential of K2509 with ion-exchange wastewater. This fermentation on account of using K2509 with wastewater results in the cost-effective, more-attractive, and environment-friendly cleaner method. As shown in Fig. 2, the trends of substrate uptake and biomass accumulation for K18 and K2509 cultivated in ion-exchange wastewater were similar to that of using tap water. However, K18 had better substrate-utilization rate and biomass-accumulation ability, although achieving lower validamycin A production than mutant K2509. These improved results might have been due to a better EMP and TCA pathway

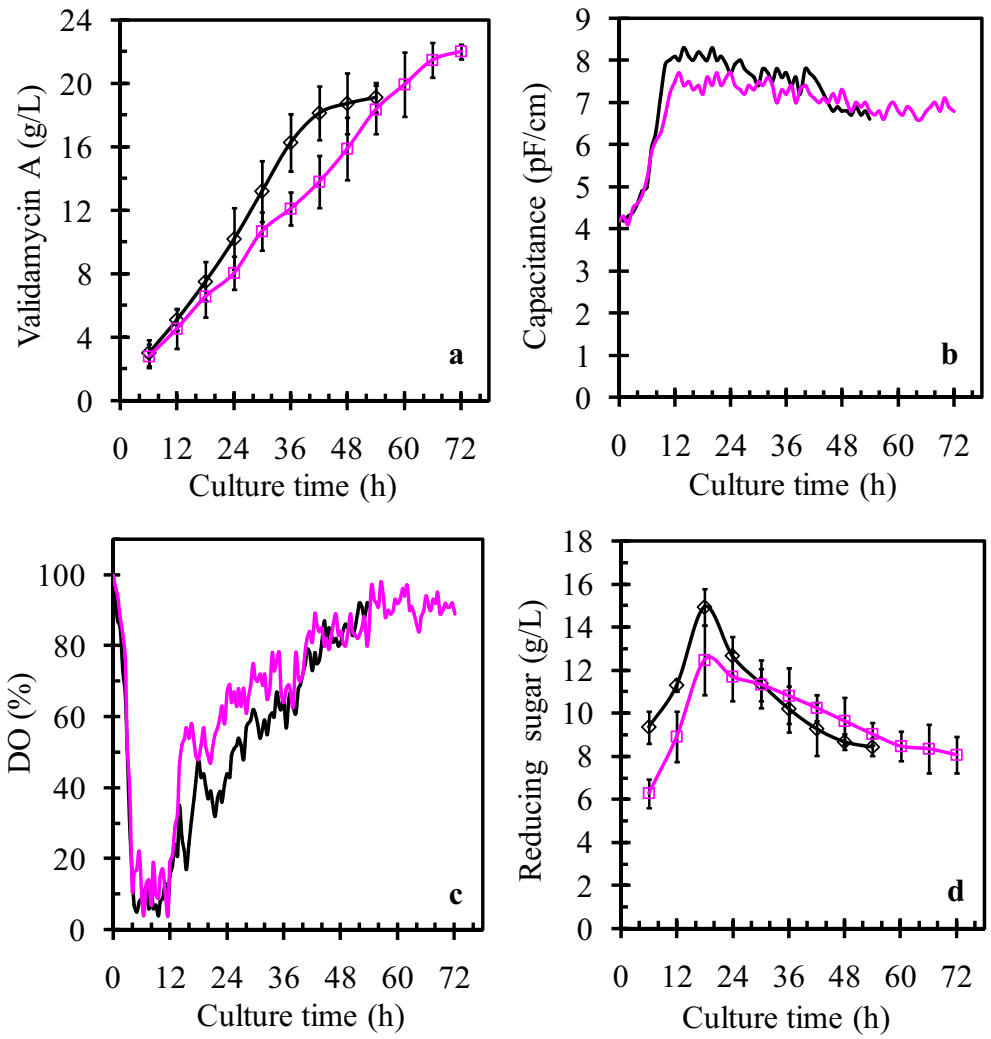

Fig. 1 Time courses of validamycin A batch fermentation with tap water in 50-L bioreactor by *S. hygroscopicus* K18 (black line) and K2509 (red line): **a** profiles of validamycin A, **b** profiles of capacitance measurement in broth, **c** profiles of reducing sugar concentration, and **d** profiles of DO

of K18 for the synthesis of biomass, meanwhile, K2509 is the trending forward PP pathway for precursor synthesis to validamycin A production (Bai et al. 2006).

Although K18 had a better productivity with respect to time, it encountered low validamycin A yield and produced more biomass and other metabolites. On other

Table 1 Test results for Ion-exchange wastewater from 4 different sample times

Date	Test items								
	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Fe^{3+} (mg/L)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	PO_4^{3-} (ms/cm)	Conductivity (ms/cm)	Amino nitrogen (mg/L)	Residual sugar (mg/L)
04-03-2016	89	66	263	665	973	4	11.39	33	337
07-04-2016	63	36	184	589	864	17	12.91	36	248
09-15-2016	102	80	213	796	738	12	6.88	54	381
12-24-2016	74	76	257	883	1037	13	21.82	47	306

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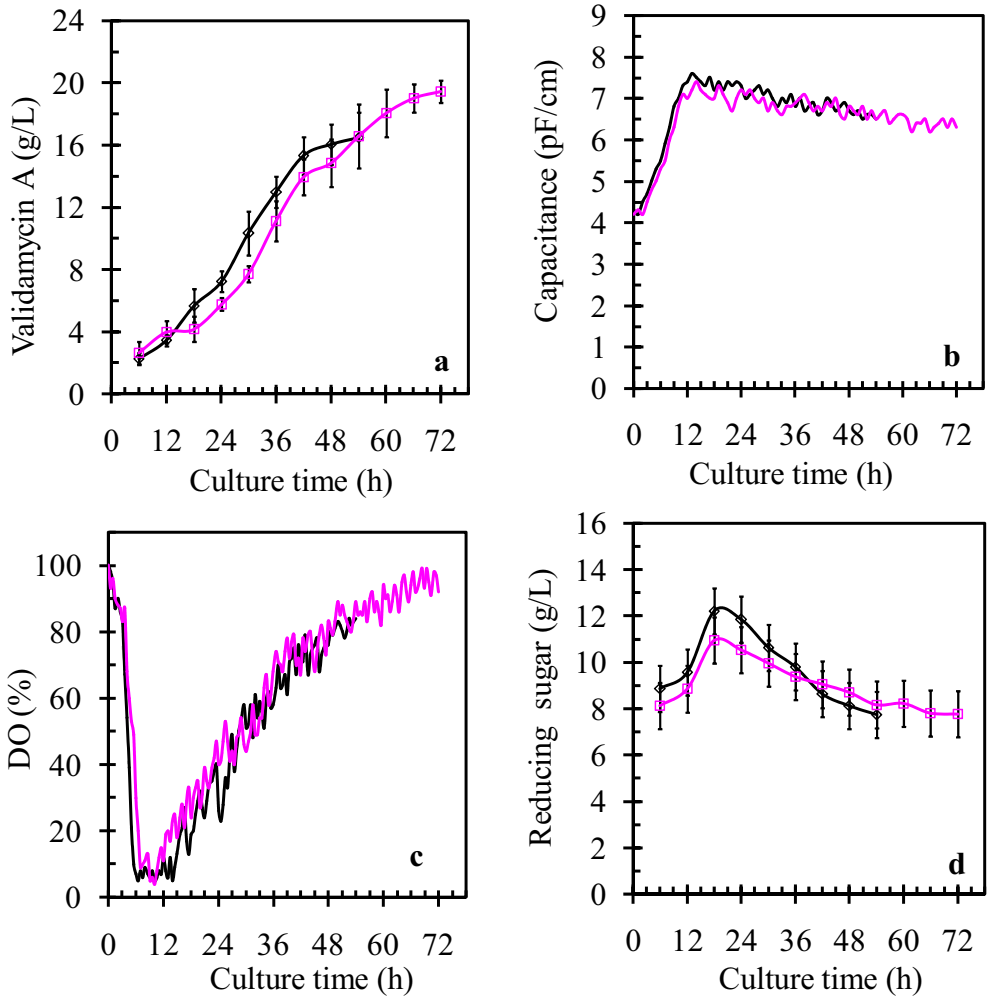

Fig. 2 Time courses of validamycin A batch fermentation with Ion-exchange wastewater in 50-L bioreactor by *S. hygroscopicus* K18 (black line) and K2509 (red line): **a** profiles of validamycin A, **b** profiles of capacitance measurement in broth, **c** profiles of reducing sugar concentration, and **d** profiles of DO

hand, K2509, although having less productivity, resulted in the better validamycin A yield with less biomass production while using wastewater. Therefore, there exists a dire need to find an alternative fermentation strategy to maximize validamycin A production. Hence, another fed-batch strategy was used to produce more validamycin A by means of wastewater (Zhou et al. 2011).

Fed-batch fermentation of validamycin A in an industrial-scale bioreactor using ion-exchange wastewater

The validamycin A production by *S. hygroscopicus* K18 and K2509 in ion-exchange wastewater is shown in Fig. 2. It could be concluded that both strains had different fermentation characteristics but K2509 had superiority for better production of validamycin A as aforesaid. While analyzing the profiles of fermentation process by K2509 in Fig. 2a, the 42 h appeared as the changing point for its metabolic process as later the productivity showed a decreasing trend. As the glucose was the key precursor for the biosynthesis of validamycin A (Zhou et al. 2011), the sugar limitation after the 42 h might be the reason for the decrement in the productivity by K2509. Therefore, a fed-batch strategy was developed to promote the production of validamycin A by K2509 in ion-exchange wastewater, and the results are shown in Fig. 3a.

After a glucose solution was fed into the broth at 36 h, the reducing sugar concentration had a significant increment from 18.33 g/L to 29.83 g/L. However, the changing point for the validamycin A's productivity

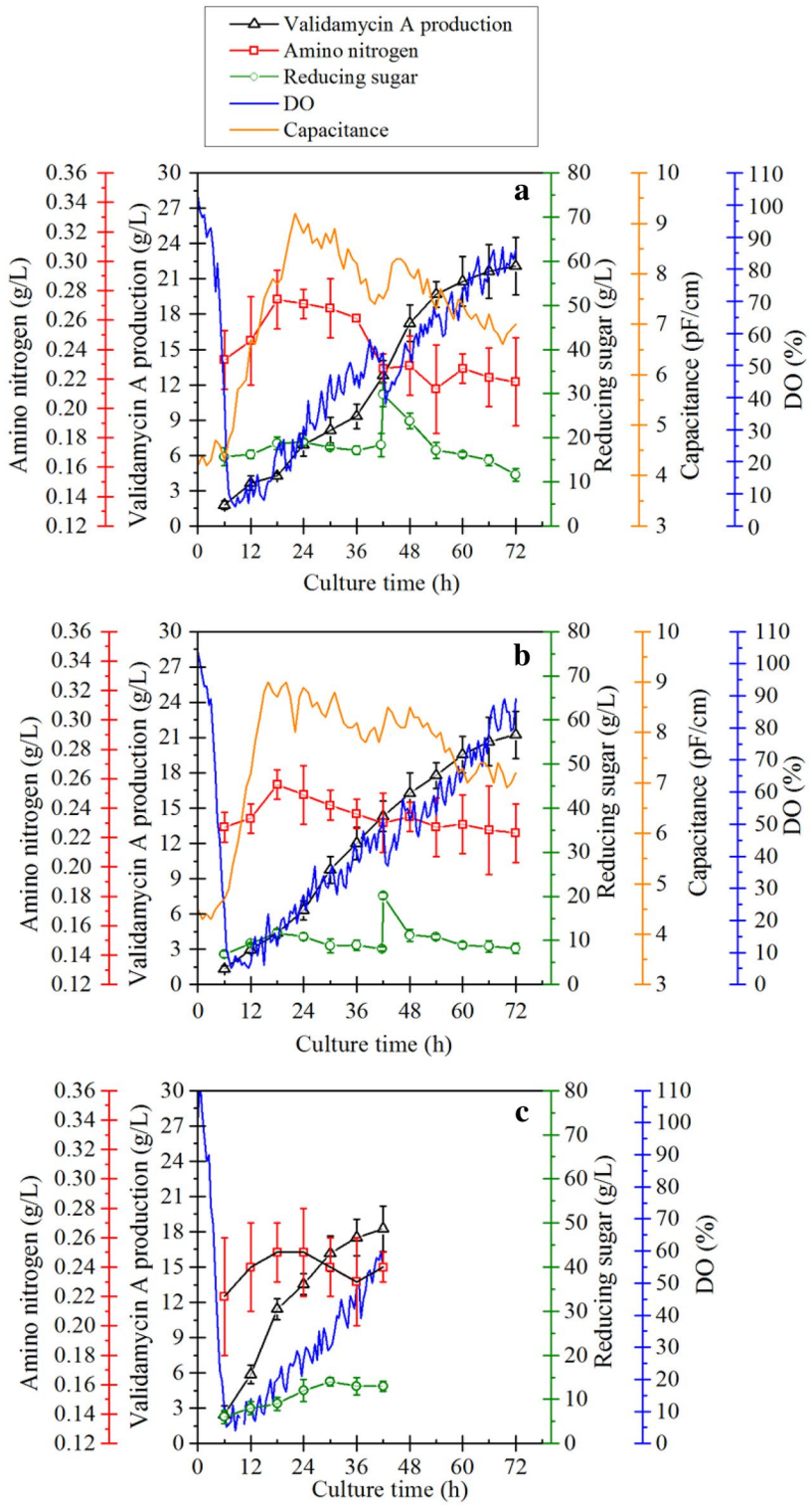

Fig. 3 Time courses of validamycin A fed-batch fermentation with ion-exchange wastewater in 50-L bioreactor (**a**) and 60 m^3 bioreactor (**b**); **c** the profile of batch fermentation with tap water in 60 m^3 bioreactor

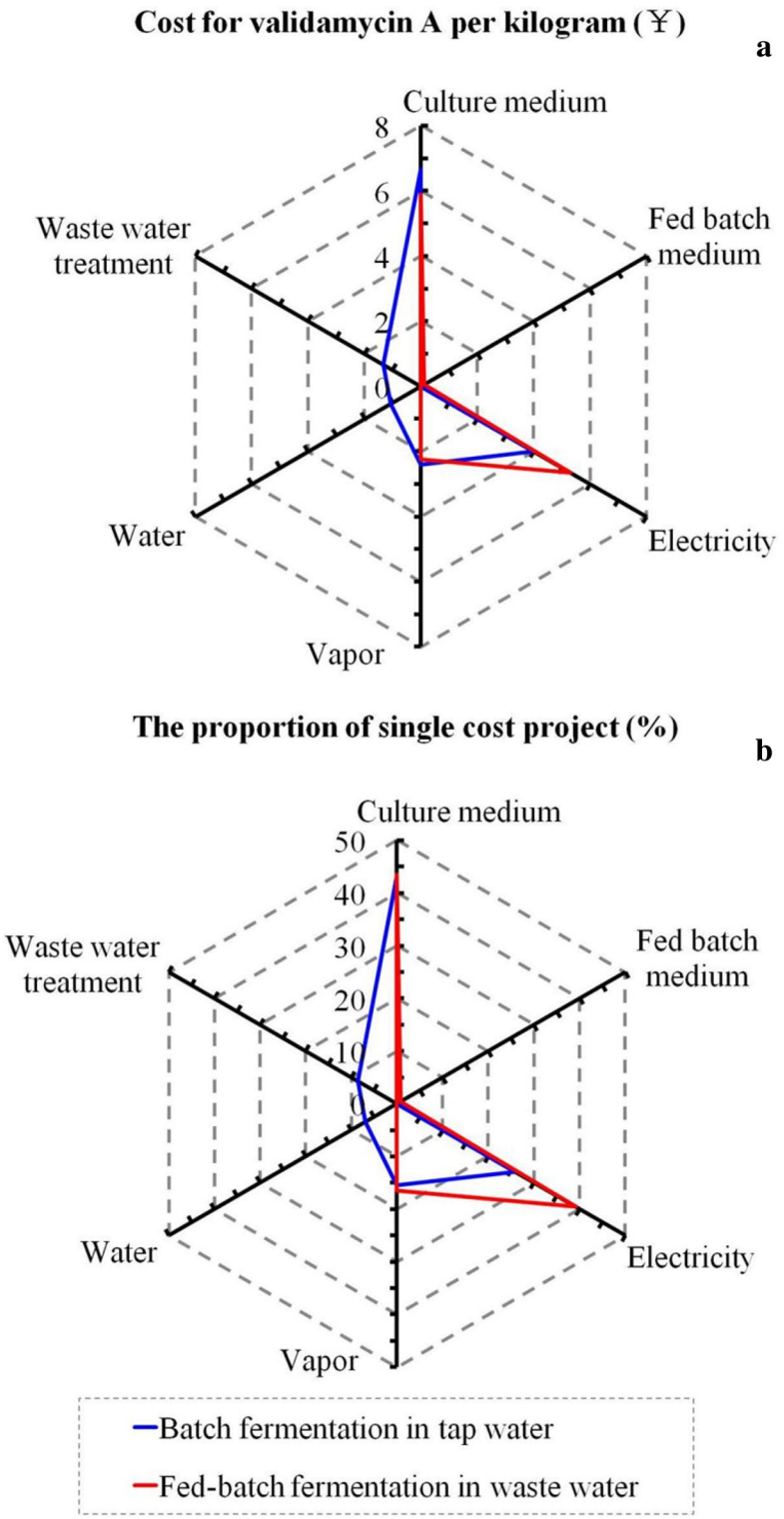

Fig. 4 The radar maps of production cost for validamycin A per gram (**a**) and proportion of single cost factor (**b**). Blue line indicates the batch fermentation in tap water by K18, red line indicates the fed-batch fermentation in ion-exchange wastewater by K2509

occurred at 42 h without glucose addition, but with glucose addition, it was not observed (Fig. 3a), and the productivity still kept at a high level till the 60 h. At 72 h, the production level reached to 22.07 g/L, indicating the fed-batch strategy is effective for increasing the production of validamycin A.

This fed-batch strategy was scaled up in a 60 m^3 bioreactor to check its feasibility on larger industrial-scale production. The dynamic profiles are shown in the Fig. 3b. The production and productivity of validamycin A were 21.23 g/L and 0.29 g/L h, respectively. The trends of other curves were similar with profiles of fermentation process executed in a 50-L bioreactor (Fig. 3a). Therefore, this culture method could be successfully and effectively applied also on industrial-scale bioreactor.

Economic evaluation for fermentation processes by using tap water and ion-exchange wastewater

Economic evaluation is one of the key steps for the feasibility study of manufacturing processes. In Fig. 4a, the industrial fermentation cost evaluation are shown, which are based on the comparison of fermentation using ion-exchange wastewater with mutated strain (Fig. 3b) and tap water with original strain (Fig. 3c). The proportions of every single cost factors are exhibited in Fig. 4b.

In batch fermentation process with tap water, the top two main cost factors were culture medium and electricity. The costs mentioned above constituted 43.10% and 25.86% of the total cost. Although vapor, tap water, and wastewater treatments are not the main costs, they also played an important role in the overall cost of validamycin A production, which accounted for 15.52%, 6.90%, 8.62% of the total cost, respectively. In fed-batch fermentation with ion-exchange wastewater, although the top two cost factors were still culture medium and electricity, which took the 43.48% and 39.13% of the total cost, nevertheless, no extra expenses were needed for water supply and wastewater treatment, sum of which was approximately 15.5% of the total cost expended in batch fermentation with tap water. Collectively, the validamycin A fermentation cost with tap water and ion-exchange wastewater were 15.46 CNY/g and 13.54 CNY/g, respectively.

With the implementation of this wastewater utilization technique, the fermentation cost could be reduced by 12.42%, which finally contributed multiple meanings to economy, society and environment. In some regions of the world, biochemical manufactures are facing serious environmental pressure from government and civil societies. In addition, high charges for industrial sewage treatment and strict discharge standards sometimes would bring the enterprise to be exposed in media and judicial platforms. Therefore, reducing the wastewater emission is not only helpful in decreasing the manufacturing cost, but also leads toward a greener and safer environment.

Moreover, it should be noticed that the fed-batch medium only occupied 0.87% of the total cost (Fig. 4b). Additionally, the cost could be further decreased with improvement exerted on culture medium, electricity, and vapor, which account for the major chunk of the manufacturing expenses. Therefore, attention should be paid toward employment of highly efficient electric machinery, using inexpensive raw materials as culture medium for reducing the capital and operating costs effectively.

Conclusions

In this paper, for first time to our knowledge, an ion-exchange wastewater-utilizing strategy was adapted for validamycin A production in plant-scale bioreactor. A new strain, *S. hygroscopicus* K2509 with good tolerance ability in ion-exchange wastewater was screened and used in the experiments. The results indicated that the validamycin A production could reach 21.23 g/L in ion-exchange wastewater by employing fed-batch strategy. Moreover, via the employment of ion-exchange wastewater, the fermentation cost of validamycin A was reduced by 12.42% that shows both economic and social significances.

Authors' contributions

WZ and XW conducted the experiments and data analysis. WZ and AM wrote the manuscript. WZ, HM, and LZ provided technical support and monitored the fermentation in plant-scale bioreactor. MG, TX, ZS, and YZ gave advices in the experimental design and data analysis. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets supporting the conclusions of this article were included in the main manuscript.

Consent for publication

All authors have read and approved the manuscript before submitting it to bioresources and bioprocessing.

Ethics approval and consent to participate
This study did not contain any studies with human participants or animals performed by any of the authors.

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