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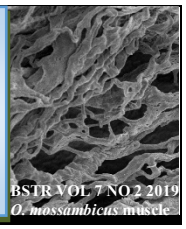

Biodegradation of Sodium Dodecyl Sulphate (SDS) by *Serratia marcescens* strain DRY6

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ABSTRACT

A bacterium capable of degrading sodium dodecyl sulphate (SDS) is characterized. Previously, the bacterium has been shown to have the capability to reduce molybdenum to molybdenum blue. In this report, we showed that almost complete degradation of SDS was observed in 6 to 10 days when the bacterium was grown on medium supplemented with SDS ranging from 0.5 to 1 g L^{-1} while higher concentrations showed partial degradation with no degradation was observed at concentrations higher than 2.5 g L^{-1}. Other detergents were also tested including Tergitol NP9, Tergitol 15S9, Witconol 2301 (methyl oleate), sodium dodecylbenzene sulfonate (SDBS), benzethonium chloride and benzalkonium chloride. However, growth can only be seen on the anionic SDBS. We also showed that the presence of metal ions such as silver, copper, cadmium, chromium, lead and mercury inhibit the ability of the bacterium to degrade SDS by 50%. Growth on SDS could not support molybdenum-reduction in this bacterium. Growth kinetic studies showed that the growth rate could be modelled using Haldane substrate inhibition kinetics with the maximum growth rate, μmax, was 0.13 h^{-1}, while the saturation constant or half velocity constant K_s and inhibition constant K_i, were 0.707 and 11.303 g L^{-1} SDS, respectively.

INTRODUCTION

Detergents are known to have damaging consequences to marine life [1–3]. In accordance with preceding reports, anionic surfactants are harmful to numerous aquatic organisms at levels which range from 0.0025 to 300 mg L^{-1} [4]. It influenced the life cycle of aquatic organisms and change in behaviour [5]. Another study reported that oyster digestive gland is sensitive to exposure to SDS causing a negative perturbation to the nutritional and metabolic functions of the oyster leading to lower survivability of oyster [6]. As more of anionic surfactants are released into water bodies, the pollutions caused by these compounds will lead to a rise in the toxic effects to invertebrates and crustaceans. Due to this, the remediation of SDS is of vital importance. Microorganisms are known for their capability to break down organic substance which includes SDS [7,8], as well as their use as bioremediation agents is financially essential for the elimination of xenobiotic contaminants. Biodegradation of anionic surfactant by the bacterium Pseudomonas sp. strain C12B under aerobic conditions was one of the earliest reports of SDS-degrading bacterium [9]. Since this study, a large number of SDS-degrading bacteria have been discovered [10–12].

Works on multiple xenobiotics-degrading or –remediating microbes are rare and since polluted sites often contain a multitude of pollutants organics and inorganics [13,14], isolation of such unique microorganisms is important. In this work we report on the characterization of a surfactant-degrading bacterium, *Serratia marcescens* strain DRY6, which also has the ability to reduce the heavy metal molybdenum to molybdenum blue [15].

MATERIALS AND METHODS

Growth and maintenance of bacterium

Serratia marcescens strain DRY6 was originally isolated as a molybdenum reducing bacterium [16] and stored in the university's culture collection unit. The basal salts (BS) medium (g L^{-1}) for bacterial growth contained the followings: KH_2PO_4, (1.36), Na_2HPO_4, (1.39), KNO_3, (0.5), $MgSO_4$ (0.01), $CaCl_2$ (0.01) and $(NH_4)_2SO_4$ (7.7). The medium also contained the following trace elements to the final concentration of 0.01 mg L^{-1}: $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, H_3BO_4, $CoCl_2.6H_2O$, $FeSO_4.2H_2O$, $CuCl_2.2H_2O$ and $Na_2MoO_4.2H_2O$. Filter-sterilized sodium dodecyl sulphate was added into the medium as a carbon source at the final concentration of 1.0 g L^{-1} [12]. The bacterium was maintained on nutrient agar plate supplemented with SDS at the same concentration and incubated at 30 °C for 5 days. Measurement of the bacterial growth was carried out using the colony count method.

Methylene blue active substance assay (MBAS)

SDS residuals were based on measuring the colour of methylene blue in the chloroform extraction method of [17] with the blue solution read at 652 nm against chloroform blank. Briefly, 100 µL of samples were mixed with 9.9 mL of deionized water in a 100 mL of separating funnels. Then 2.5 mL of methylene blue solution was added followed by the addition of 1 mL of chloroform. The extraction was commenced with the funnel shaken vigorously for 15 s. Two layers will be formed after the mixture was left for 20 min. The chloroform layer was taken out and placed into a second funnel. The process of extraction was repeated three times using 1 mL of chloroform per extraction. The chloroform extracts were then combined in the second funnel and the funnel shaken vigorously after adding 5 mL of wash solution for 15 s. The organic chloroform layer was drawn off into a 10 mL volumetric flask. The extraction process was repeated twice using 1 mL of chloroform. Ultimately, every one of the extracts was put together and diluted to the 10 mL mark by adding more chloroform.

Kinetic studies

The profile of numerous biomass growth rates can be utilized in obtaining kinetic parameters from batch tests. Bacterial dry weight determination was done by plotting dry weight of biomass per litre versus bacterial count (CFU mL^{-1}). The specific growth rate coefficient (μ) can be gotten at each of the initial diesel concentration by plotting In X (bacterial dry weight) vs. time. A nonlinear curve will be obtained when these values were plotted against substrate concentration. As opposed to the classical Monod model the Haldane model is the model of choice in modelling growth under substrate inhibition conditions. The inhibition models are as follows;

$$\mu_{\max}\frac{S}{K_s+S} \qquad [18]$$

$$\mu_{\max}\frac{S}{S+K_s+\frac{S^2}{K_i}} \qquad [19]$$

where, μ, μmax, S, K_s and K_i, are the specific growth rate (h^{-1}), maximum specific growth rate (h^{-1}), substrate concentration (% (v/v) or mg L^{-1}), half-saturation constant (mg L^{-1}), inhibition constant (mg L^{-1}), respectively.

The constants were obtained by running data on GraphPad Prism using the Michaelis-Menten substrate inhibition kinetics and replacing the resultant enzyme kinetic constants with SDS degradation kinetics constants.

Statistical analysis

Statistical software (Graphpad Prism version 3.0) was used to analyse the results. Values are taken as means ± SE for three replicates. Comparison between groups was performed using a one-way analysis of variance with post hoc analysis by Tukey's test or the Student's t-test [20]. $P < 0.05$ was considered statistically significant.

RESULTS

Optimization of temperature

The study of bacterial optimum temperature for growth on xenobiotics would be very useful for bioremediation purposes. This is especially important with bioaugmentation experiment since large-scale growth of the bacteria can be carried out under closed optimum conditions. The effect of temperature on the SDS degradation efficiency by *S. marcescens* strain DRY6 was studied at temperatures ranging from 10 to 50 °C. Growth rate of *S. marcescens* strain DRY6 on SDS was shown to be the highest at 35 °C ($p<0.05$). Growth decreased rapidly at incubation temperature above 40 ºC and almost no growth observed at temperatures higher than 50 °C (**Fig. 1**).

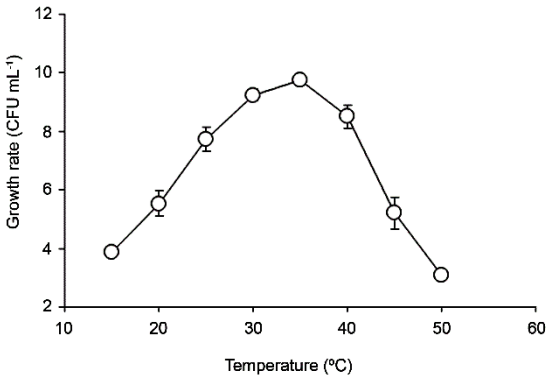

Fig. 1. The effect of temperature on the growth of *S. marcescens* strain DRY6. Data is mean ± standard error (n=3).

The effects of nitrogen source on growth

Nitrogen source is a crucial component that has an effect on the growth of microorganisms. Consequently, detection of the best nitrogen source and its particular optimum concentration for growth could greatly assist in creating successful bioremediation approach [21]. Different nitrogen sources such as ammonium sulphate, ammonium chloride, potassium nitrite and potassium nitrate were tested at 0.1% (w/v) in BS media supplemented with SDS as the sole carbon source to study their effects on bacterial growth. Our results have shown that *S. marcescens* strain DRY6 growth rate was the highest when ammonium sulphate is the sole nitrogen source ($p<0.05$) (**Fig. 2**). The optimal concentration of ammonium sulphate was at 2 g L^{-1}.

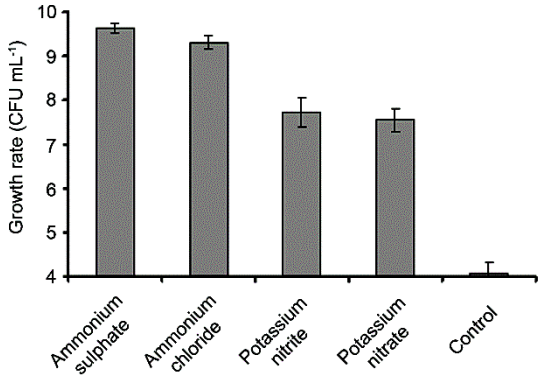

Fig. 2. The effect of various nitrogen sources on the growth of *S. marcescens* strain DRY6. Data is mean ± standard error (n=3).

Optimization of pH

As pH strongly affects bacterial growth, the maintenance of pH in the medium is vital. Once the optimum pH for the bacterial growth is obtained, this can help in designing effective bioremediation strategy [22]. Our results showed that *S. marcescens* strain DRY6 has the best growth rate in the pH range from 6.5 to 7.5 (**Fig. 3**).

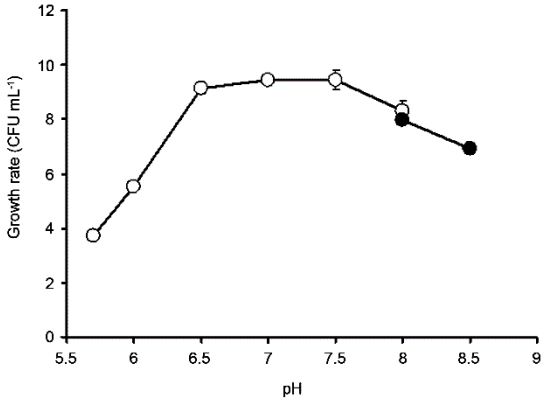

Fig. 3. The effect of pH on the growth of *S. marcescens* strain DRY6 using an overlapping buffer system consisting of phosphate (○) and carbonate (●). Data is mean ± standard error (n=3).

The effects of sodium dodecyl sulphate concentrations on growth

Sodium dodecyl sulphate as being the lone supply of carbon is required in big amounts as carbon is the fundamental structural unit of all organic substances. The bacteria may also be killed by the stripping of the lipopolysaccharide outer layer by SDS especially in Gram negative bacteria leading to cell death [1,23]. We showed that *S. marcescens* strain DRY6 was able to utilize SDS as a sole carbon source. We observed the growth rate of *S. marcescens* strain DRY6 on a series of different concentration of SDS and the highest growth rate was recorded at the concentration between 1-1.5 g L^{-1} ($p<0.05$). *S. marcescens* strain DRY6 exhibited lower growth rate at SDS concentration between 2 to 3 g L^{-1} and growth was strongly inhibited at 5 g L^{-1} (**Fig. 4**).

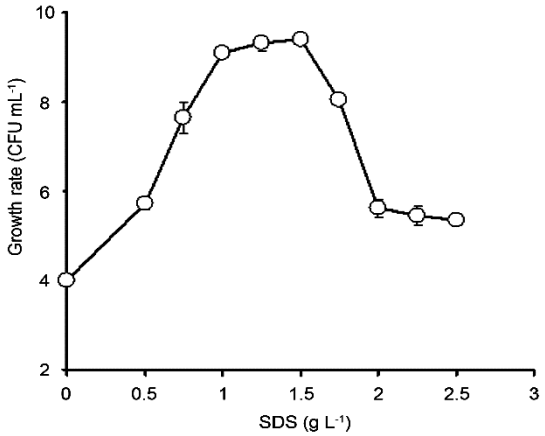

Fig. 4. The effects of sodium dodecyl sulphate concentrations on the growth of *S. marcescens* strain DRY6. Data is mean ± standard error (n=3).

Growth on other anionic and cationic surfactants

Other heavily used commercial surfactants, Tergitol (Sigma) and sodium dodecylbenzene sulfonate (SDBS) (Sigma) were tested at the concentration of 1 g L^{-1} for their ability to support the growth of *S. marcescens* strain DRY6. Tergitol belongs to a surfactant category called ethoxylated surfactant. This surfactant consisted of 15% of the U.S. surfactant market [24]. SDBS, most often used in laundry detergent is a linear primary alkylbenzene sulfonate. **Table 1** showed that only the anionic SDBS supported the growth of *S. marcescens* strain DRY6.

Table 1. Growth of *S. marcescens* strain DRY6 on detergents.

Detergent	Type	Growth
Tergitol NP9	Nonionic	-
Tergitol 15S9	Nonionic	-
Witconol 2301 (methyl oleate)	Nonionic	-
Sodium dodecylbenzene sulfonate	Anionic	+
Benzethonium chloride	Cationic	-
Benzalkonium chloride	Cationic	-

SDS-degradation at different initial concentrations

The ability of *S. marcescens* strain DRY6 to degrade SDS was further tested on a series of different concentration of SDS. We showed that the growth of *S. marcescens* strain DRY6 was completely inhibited at 2 g L^{-1} of SDS (**Fig. 5**). Almost complete degradation of SDS was observed after 6, 8 and 10 days of incubation at 0.5, 0.75 and 1 g L^{-1} of SDS, respectively, while higher concentration showed partial degradation with no degradation, was observed at 2.5 g L^{-1} SDS after 10 days of incubation.

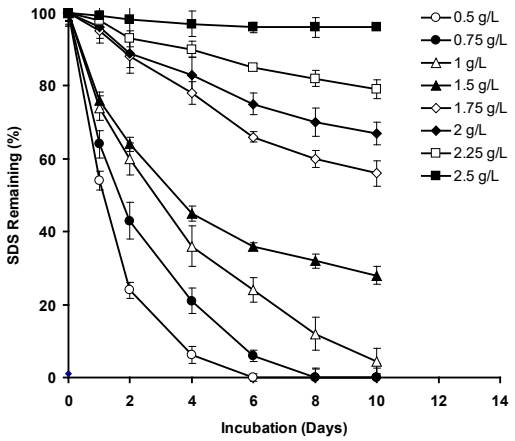

Fig. 5. SDS degradation by *S. marcescens* strain DRY6 at various initial concentrations. Data is mean ± standard error (n=3).

Growth kinetics studies

The CurveExpert Professional software (Version 1.6) was fitted to two kinetic models of growth i.e- Monod and Haldane using custom equations algorithm which minimizes the sums of square of residuals. A correlation coefficient value of 0.99 for the Haldane model indicates it fits the curve while a low correlation coefficient value of 0.34 for the Monod model indicates poor-fitting (**Fig. 6**). The specific growth rate μ was discovered to rise as the substrate concentration was increased but it reached a peak value followed by a slow decrease indicating substrate inhibition. The calculated μmax or maximum growth rate was 0.13 h^{-1} while the saturation constant K_s or half velocity constant was 0.707 g L^{-1} SDS. The inhibition constant K_i was 11.303 g L^{-1} SDS.

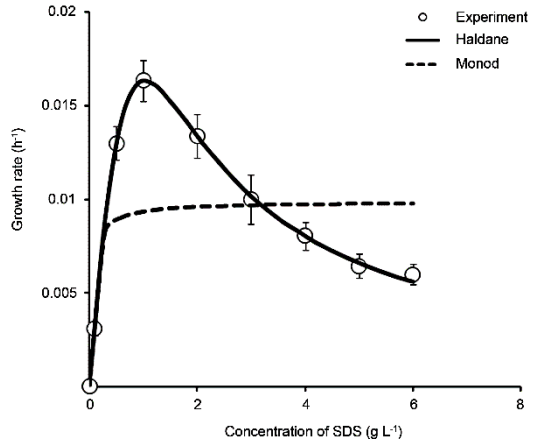

Fig. 6. Growth kinetics of *S. marcescens* strain DRY6 on SDS. Data represents mean ± SEM (n=3).

Growth of *S. marcescens* strain DRY6 on heavy metals

To determine the potential ability of *S. marcescens* strain DRY6 to utilize heavy metals, we tested the growth of *S. marcescens* strain DRY6 on various heavy metals including zinc (Zn), silver (Ag), nickel (Ni), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and mercury (Hg). We showed that *S. marcescens* strain DRY6 has a high growth rate on Zn and Ni (**Fig. 7**). Growth on silver and chromium was reduced by 70% while there was 80% growth reduction observed on Cd, Cu and Pb. Growth on Hg was severely inhibited.

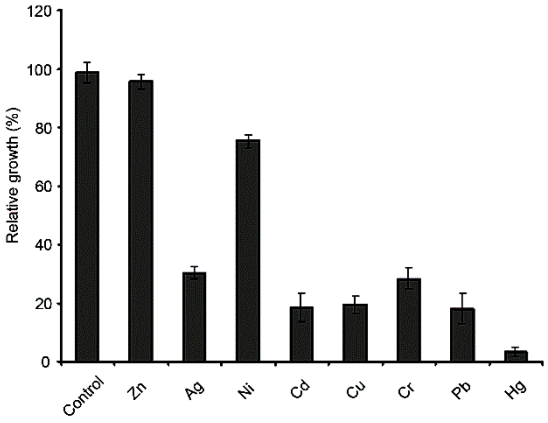

Fig. 7. The effect of various heavy metals on the growth of *S. marcescens* strain DRY6. Data is mean ± standard error (n=3).

DISCUSSION

Sodium dodecyl sulphate (SDS) is one of the components used in detergent formulations [25]. It is used widely in industrial and household applications [26]. Problems arise when wastewater from industrial and household containing SDS is discharged into the river thus polluting the environment. Many treatments have been used to treat surfactants in wastewater including microorganisms which have the ability to degrade surfactants [27]. The first work describing the ability of bacteria to degrade SDS was reported by [9].

In this study, we investigate the ability of *S. marcescens* strain DRY6 to degrade SDS. *S. marcescens* strain DRY6 was isolated from soil near the State Museum in the city of Taiping, state of Perak, Malaysia and was previously shown to have the capability to reduce molybdenum to molybdenum blue [15]. We showed that *S. marcescens* strain DRY6 was able to degrade SDS. The variety of SDS-degrading bacteria reported in the literature includes *Acinetobacter calcoaceticus* and *Pantoea agglomerans* [28], *Pseudomonas betelli* and *Acinetobacter johnsoni* [29], *Klebsiella oxytoca* [30] as well as *Burkholderia* sp., and *Serratia odorifera* [31,32].

The spectrum of temperature affecting SDS degradation by microbes falls under common extremes ranging from polar to tropical. Examples include the SDS-degrading bacteria *Pseudomonas* Strain C12B [9] that degrade SDS optimally at 30 °C. Marchesi et al. reported that the optimum temperature of mesophilic *Pseudomonas* sp. is at 25 °C [33]. Roig et al. showed that *Comamonas terrigena* strain N3H required optimum growth at 28 °C [10], whereas *Citrobacter braakii* and *Delftia acidovorans* strain SPB1 showed optimum growth at 30 °C [11,12]. In contrast, psychrotolerant SDS-degrading bacteria can carry out degradation at much lower temperatures (less than 10 °C) [34]. The preference for neutral to slightly alkaline pH for the degradation of SDS was demonstrated by several other SDS-degrading bacteria. *Klebsiella oxytoca* strain DRY14 exhibits an optimum growth at pH 7.25 [30]. *Delftia acidovorans* strain SPB1 showed an optimum pH for growth on SDS at 7.2 [11]. *Citrobacter braakii* required pH 7.0 [12] whereas *Comamonas terrigena* strain N3H required pH 7.4 [10]. The growth of *S. marcescens* strain DRY6 decreased significantly at pH 9.5, presumably due to extreme alkaline conditions.

The ability of bacteria to regulate their cytoplasmic pH allows them to tolerate a certain range of pH [35]. However, extremely acidic and alkaline conditions affect the state of ionization of active sites of enzyme and lead to changes in the electronic configuration of the active site eventually preventing substrate binding. This is translated to a loss of activity [35].

The study of pH optimal is important for two reasons. The first is for mass production of the bacterium in bioaugmentation exercise and the second is to assess whether pH adjustment of soil in polluted sites to match optimal growth or degradation of the bacterium is needed. The use of ammonium sulphate as a nitrogen source is consistent with previous reports by Dhouib et al. [12] and Shukor et al. [30]. Other surfactant degraders like *Citrobacter braakii* required 7.7 g L^{-1} ammonium sulphate [12] whereas *Comamonas terrigena* strain N3H showed an optimum growth at 5.4 g L^{-1} ammonium nitrate [10].

The ability of *S. marcescens* strain DRY6 to assimilate SDS for growth falls under common tolerable SDS concentration range reported in the literature. The maximum degradation capacity by bacteria reaches a limit often coinciding with the critical micelle concentration (CMC) of SDS at 2.34 g L^{-1}. *S. marcescens* strain DRY6 shows that at the tolerable concentration of 2 g L^{-1}, approximately 90% of SDS was degraded after 8 days and cellular growth had reached equilibrium. However, a longer lag period of approximately three days was observed before the bacterial growth started to increase concomitantly with a reduction in SDS concentration implying that adaptation of the bacteria to different carbon source. Margesin and Schinner [34] reported that their consortia of microbes are able to degrade 0.5 to 1 g L^{-1} SDS in 4 days at 10 °C [34]. The tropical isolate *Klebsiella oxytoca* strain DRY14, isolated from a detergent-polluted site, does not exhibit any lag phase during its degradation of 2 g L^{-1} SDS, implying that the genes for detergent degradation are quickly expressed upon contact with a detergent such as SDS [30]. The ability of *S. marcescens* strain DRY6 to degrade SDBS together with SDS is another advantage as SDBS is a much hardier substrate than SDS. SDBS-degrading bacteria have been reported in the literature [36,37] while there is very limited information on the use of Tergitol, Witconol and the cationic detergents benzethonium chloride and benzalkonium chloride as carbon sources.

There are few data on SDS-degradation and utilization kinetics in the literature. Other substrate-inhibiting models that have been employed include the Andrew and Tessier model with Andrew reported to be the best model as reported by Khleifat et al. giving μmax, K_s and K_i, values of 0.26 h^{-1}, 0.6 g L^{-1} and 1.5 g L^{-1}, respectively [32]. The μmax value obtained in this work is lower than values reported for a co-culture mixture of several SDS-degrading bacteria such as *Burkholderia* sp., *Acinetobacter calcoaceticus*, *Klebsiella oxytoca* and *Serratia odorifera* that ranges between 0.21 and 0.26 h^{-1} [31,32].

The ability of microorganisms to grow on heavy metals on easily assimilable substrates has been reported. For instance, *Pseudomonas putida* has been reported to be able to tolerate high concentration of heavy metals such as Cd, Zn and Pb [38,39]. *Paenibacillus* sp. was shown to have high sensitivity against Cu while *Bacillus thuringeinsis* has a high sensitivity against Cd and Zn [40]. However, heavy metal tolerant SDS-degrading bacteria or studies on the effect of heavy metals on SDS degradation are limited. Hence, this study offers novel data for comparison on SDS-degrading bacteria isolated in the future. The ability of this strain to degrade SDS could not be coupled to molybdenum reduction as SDS is probably inhibitory towards the molybdenum-reducing enzyme activity in this strain.

CONCLUSION

In conclusion, this is the first report of a molybdenum-reducing bacterium having the ability to degrade SDS. The characteristics of the growth on SDS have been explored and show variation in optimal conditions compared to published database. Our attempt to use other detergents as a source of carbon was not successful with the exception of SDBS. The bacterium could completely remove SDS after 10 days at 1 g L^{-1}. We also showed that the presence of metal ions such as silver, copper, cadmium, chromium, lead and mercury inhibit the ability of the bacterium to degrade SDS. Growth on SDS could not support molybdenum-reduction in this bacterium. Growth kinetic studies showed that the growth rate could be modelled using Haldane substrate inhibition kinetics. The characteristics of this bacterium make it suitable for bioremediation of SDS-polluted environment.

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