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Potato catalase-promoted rapid release of oxygen from hydrogen peroxide used in aquaculture

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Abstract
Under the drastic condition of dissolved oxygen seriously lacked in aquaculture water, it is critical to rapidly release oxygen by throwing oxygenation reagent into water, for aquatic animals surviving. Here, the adduct sodium sulfate-hydrogen peroxide-sodium chloride as oxygen-released agent was catalyzed by crude catalase solution (CCS) from potato starch process to rapidly increase molecular oxygen. In 500 mL water, the adduct catalyzed by 2 mL CCS can increase dissolved oxygen by 5.16 $mg \cdot L^{-1} \cdot h^{-1}$ and give almost no impact on pH and redox potential, but the commercially available sodium percarbonate only brings dissolved oxygen by 0.68 $mg \cdot L^{-1} \cdot h^{-1}$, and causes pH value up to 10 and redox potential down to 70 mV, when hydrogen peroxides in the two oxygen-released agents all are at 0.043 $g \cdot L^{-1}$. This and further researches display that CCS not only has high catalysis but also is friendly to water environment.

Keywords: Dissolved oxygen, catalase, catalysis, hydrogen peroxide, waste water

1. Introduction
In aquaculture ponds or intensive culture systems, not only fish and most other aquatic animals breathe through gills that absorb molecular oxygen (dissolved oxygen) from the water in which they live, but also phytoplankton and microbial as well as organic compounds all consume a large amount of oxygen, for the water eutrophication induced by decomposition of uneaten feed, feces and metabolic excretion from aquaculture animals [1, 2]. As a result, the dissolved oxygen naturally diffused from atmosphere to water or from photosynthesis of aquatic plants and algae cannot meet the requirement of them in growth and survival, and thus the extra oxygen needs to be artificially supplemented to water body [3]. Especially under the drastic condition such as sultry weather before thunderstorm, dark background at night or narrow water body during intensive live fish transportation, the low air pressure and the invalid photosynthesis lead to the concentration of dissolved oxygen dramatically dropped in water body, and then fish and shrimp have to let heads floating over surface of water for gulping air [4-6]. At this moment, to avoid them dying of asphyxia, the adequate oxygen needs rapidly diffused into water.
The common method for aeration in aquaculture water is to mix air with surface water by variously designed aerators, so as to accelerate diffusion of oxygen from air to water [2, 7, 8]. However, the mechanical aeration is invalid under the emergency conditions as mentioned above, for that the aerobic machineries are located in finitely fixed positions and thus the directly mixed oxygen is only diffused in local surface water layer where dissolved oxygen takes a long time to diffuse around, depending on concentration difference of dissolved oxygen [9]. The other strategies, e.g. biological and newly developed approaches [10-12], generally are suitable for increasing oxygen in the aquatic water under non abnormal conditions. Hitherto, the most effective way to rapidly increase dissolved oxygen in water body is relied on some availably chemical reagents, i.e. peroxides such as calcium peroxide (CP) [13, 14], hydrogen peroxide (HP) [5, 15], and some HP adducts, e.g. sodium percarbonate (SP) [16] and sodium sulfate-hydrogen peroxide-sodium chloride (SHS) [17], etc.
Among these oxygenation reagents, HP is unstable and inconvenient in storage and transportation [18]. And CP and the salts motioned above are relatively stable, but they release oxygen slowly in water, and CP and SP would result in water being alkaline [19, 20], which is harmful to growth of aquatic animals, especially those farmed in alkaline water environments [21, 22].

Comparatively, SHS brings about no increase in pH value of water and gives no other hazardous effects on water, that is to say, it is an alternative oxygen-released agent used for rapidly increasing dissolved oxygen in aquaculture ponds under emergency situations [23, 24], provided that there is a way to effectively promote SHS releasing oxygen. Then it is imagined to accelerate decomposition of HP in SHS by catalase as a biocatalyst instead of chemical catalysts, ferrous salts commonly used in Fenton reaction [25], on account of the enzymatic advantages of enzymes in high catalytic efficiency and environmental friendliness [26]. Considering the economic feasibility, here the wastewater solution from potato starch process is selected as a usable bioresource in which catalase is rich [27]. The solution is simply processed into crude catalase solution (CCS) and used to promote SHS to decompose and release oxygen faster in water body (Figure. 1).

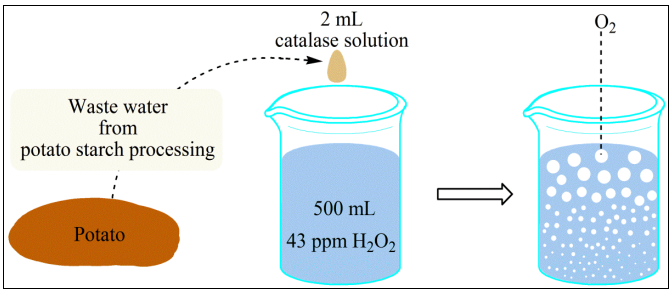


Fig 1: Dissolved oxygen rapidly increased by decomposition of hydrogen peroxide promoted by catalase solution

2. Materials and Methods

2.1. Apparatus and materials

Dissolved oxygen concentrations were measured by a portable dissolved oxygen analyzer (HQ30d), pH values of solutions were probed by a pH meter (HI98190), oxidation-reduction potentials of water samples were recorded on a redox potentiometer (HI98120), enzymatic activities were analyzed on a ultraviolet-visible (UV-vis) spectrophotometer (T6 new century), fresh potatoes were homogenized in a soybean milk machine (DJ13E-Q1), and potato-processed filtrate was further separated in a speeding centrifuge (Avanti JXN-26).

Potato sample was bought from Tianjin Hongqi Agricultural Trade Market and Coomassie Bright Blue G-250 Kit was ordered from Nanjing Jiancheng Bioengineering Institute. In experimental, the concentration of HP solution was 30 %, the percent contents of HP in products SP ($2Na_2CO_3 \cdot 3H_2O_2$) and SHS ($4Na_2SO_4 \cdot 2H_2O_2 \cdot NaCl$) were 28.7% and 9.5%, respectively. And the other reagents all were AR grade chemicals.

2.2. Preparation of CCS from potato starch processing process

Referring performance in the literature [28], 400 g of cleaning washed fresh potatoes were cut into pieces of blocks (about 1.0 cm^3) and homogenized together with 400 mL of phosphoric acid buffer with molar concentration 0.05 $mol \cdot L^{-1}$ and pH 7.2. The obtained potato slurry was filtrated with six layers of gauze and the filtrate was set at 4 ℃ for 2 h, thereafter the filtrate was further separated by centrifugation at 4000 rpm and the supernatant as CCS was kept at 4 ℃ for the later experimental.

2.3. Measuring protein, activity and kinetics of CCS

The content of protein in CCS was determined by Coomassie Bright Blue G-250 Kit. In a ratio of 1:4, the dye reagent solution was dissolved in water before use. The standard protein sample was diluted down to a useful concentration 0.563 $g \cdot L^{-1}$. 50 μL double distilled water and 50 μL standard protein solution or sample solution were mixed in 3 mL of diluted G-250 solution and analyzed at 595 nm. Based on the measurement results and the corresponding calculation method [29], the acquired content of protein in sample was 0.31%.

By UV-vis method [30], the activity of catalase in sample solution was determined after the standard curve between the mass of HP and the absorbance of HP solution was made. To the control tube, 1.5 mL phosphoric acid buffer (0.1 $mol \cdot L^{-1}$, pH 7.8), 0.1 mL CCS, 0.2 mL distilled water, 1.0 mL sulfuric acid solution (8%) and 0.2 mL HP solution (0.15 $mol \cdot L^{-1}$) were added in order and measured at 240 nm. To the sample tube, the aforesaid solutions other than sulfuric acid were added and kept for 4 min, and again 1.0 mL sulfuric acid solution (8%) was mixed to terminate enzymatic reaction and measured at 240 nm. Here 1 unit of catalase activity (IU) was defined as the volume of CCS (mL) consumed in conversion of 1 mmol HP in 1 min. Again the activities of CCSs were periodly measured at different temperature and pH value. At the optimal temperature and pH value, the initial activities of CCSs were measured by employing different concentration of HP after catalyzing for 4 min.

2.4. Oxygen-released reactions of several systems

At 22± 1℃, nine test groups were set up and tabbed by number 1-9 samples for testing, and all were repeated for three times in operation and the average values were taken. For every one, 500 mL of distilled water was taken as solvent in which, for 1-6 samples, 0.453 $g \cdot L^{-1}$ SHS solutions were prepared by dissolving solid SHS and then CCS was added by 0, 0.5, 1, 2, 4, 6 mL, respectively. For the seventh, 0.043 $g \cdot L^{-1}$ HP was prepared by adding 30% HP solution. For the eighth, 0.15 $g \cdot L^{-1}$ SP was prepared by dissolving solid SP. For the ninth, it was set as a blank control in which nothing was added. After necessary mixing performance, they were tested periodly by dissolved oxygen analyzer, pH meter and redox potentiometer.

2.5. Oxygen-released reactions of SHS by re-adding catalase

Seven test groups were set up and tabbed by number 1-7 samples for testing, and all were repeated for three times in operation and the average values were taken. For number 1-7 samples, 500 mL of distilled water was taken as solvent in which, for 1-6 samples, 0.453 $g \cdot L^{-1}$ SHS solutions were prepared by dissolving solid SHS and then CCS was added by 0, 0.5, 1, 2, 4, 6 mL, respectively. For the seventh, it was set as a blank control in which nothing was added. After necessary mixing performance, they were tested periodly by dissolved oxygen analyzer, pH meter and redox potentiometer. For the samples with catalase, again they were added equal volume of CCSs after 6 h.

2.6. Oxygen-released reactions of SHS Catalyzed by catalase, Fe^{2+}, and Fe^{3+}

Five test groups were set up and tabbed by number 1-5 samples for testing, and all were repeated for three times in operation and the average values were taken. For number 1-5 samples, 500 mL of distilled water was taken as solvent in which, for the first sample, it was set as a blank control in which nothing was added. For 2-5 samples, 0.453 $g \cdot L^{-1}$ SHS solution was prepared by dissolving solid SHS. In the second,

no CCS was added, and in the third, there was 3 mL of CCS added. In the fourth, 8 mL of 2.78 g·L^{-1} $FeSO_4·7H_2O$ solution was added, and in the fifth, there was 8 mL of 2.78 g·L^{-1} $FeCl_3·6H_2O$ added. After necessary mixing performance, they were tested periodly by dissolved oxygen analyzer, pH meter and redox potentiometer.

2.7. Oxygen-released reaction of SHS Catalyzed by catalase in aquaculture water

Firstly, the enough aquaculture water was taken and the part was filtrated through filter membrane (0.45μm). For filtered aquaculture water, four test groups were set up and tabbed by number 1-4 samples for testing, and all were repeated for three times in operation and the average values were taken. The sample 1 was 500 mL of filtrated aquaculture water used as a blank control, the sample 2 was 0.453 g·L^{-1} solution of SHS in 500 mL filtrated aquaculture water, the sample 3 was 0.453 g·L^{-1} solution of SHS in filtrated aquaculture water with extra 3 mL CCS, and the sample 4 was 0.15 g·L^{-1} solution of SP in 500 mL filtrated aquaculture water. For unfiltered aquaculture water, also four experimental samples were gotten ready by employing unfiltered aquaculture water to replace filtered aquaculture water in the four samples aforesaid, and numbered by 1′-4′. After necessary mixing performance, all samples were tested periodly by dissolved oxygen analyzer, pH meter and redox potentiometer.

3. Results and Discussion

3.1. Properties of potato catalase

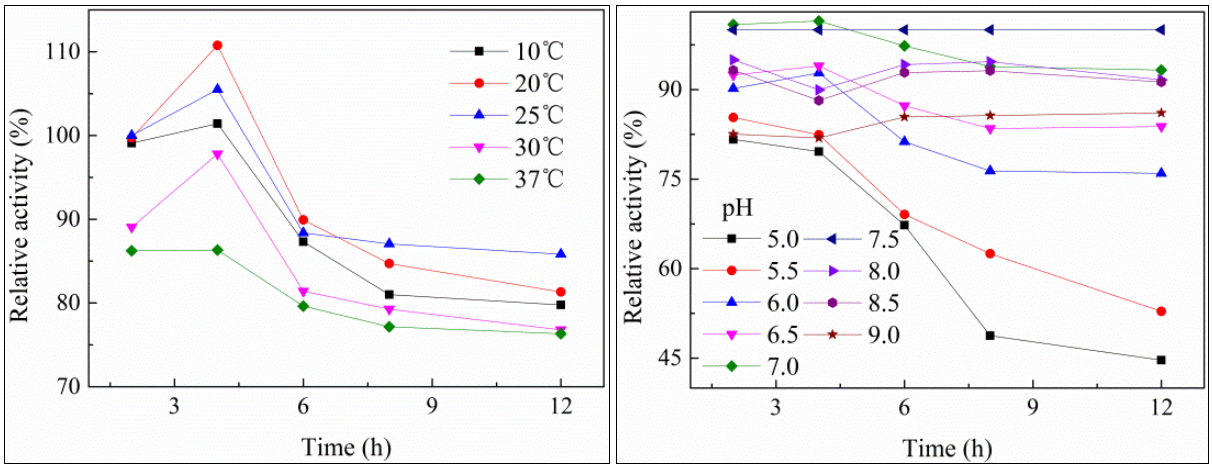
Here CCS is obtained by the performance similar to potato starch process and tested by Coomassie Brilliant Blue G-250 Kit to know that the solution contains 0.31% of protein. The average activity of CCS at 25℃ is 0.176 IU·mL^{-1} based on the definition that 1 IU is the volume of CCS consumed in conversion of 1 mmol HP in 1 min.

If the initial activity of CCS at 25℃ was considered to be 100% and then the relative activities of CCS at different temperatures and times could be exhibited in Figure. 2a. It is shown that CCS behaves good activities at the temperature from 10 to 25℃ and still has more than 80% activity remained even if after 12 h. Though the activity has some decrease when temperature set at 30-37℃, it at least keeps 77% activity after 12 h. These illustrate that the catalase has relatively stable activity at the temperature 22-28℃ common employed in aquaculture [31]. If the activity of CCS at pH 7.5 was set to be 100%, then the relative activities of CCS at various pH values and times were shown in Figure. 2b. It is found that CCS activity is very stable at pH 6-9 and declines somewhat beyond the pH range. At pH 5, the activity remains only 45% after 12 h. It is obvious that CCS is suitable for the aquaculture environment with pH value around 7.5 [22].

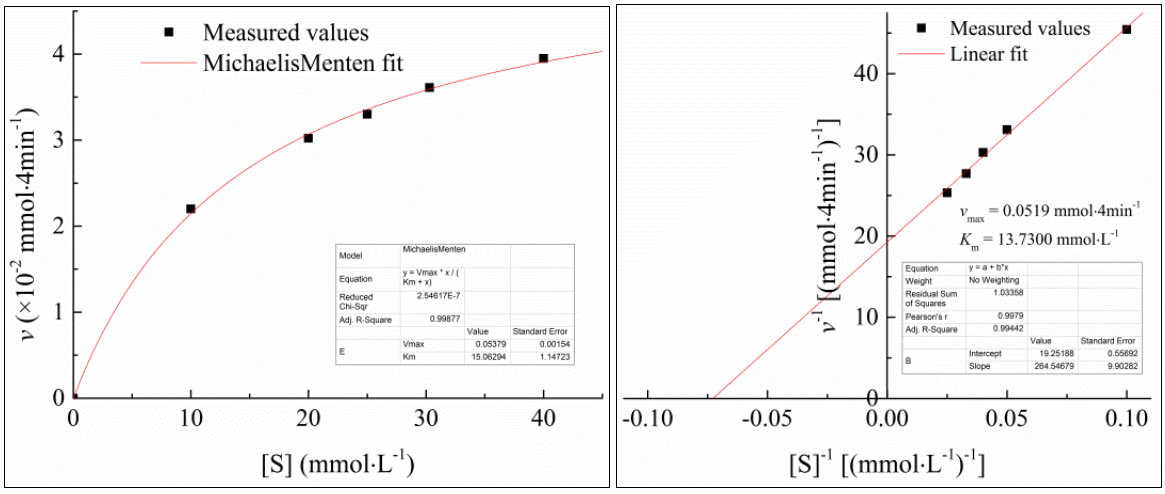
Under the better conditions 25℃ and pH 7.2, the reaction kinetics of CCS-catalyzed HP decomposition have been measured and the kinetic curve is obtained by Michaelis Menten fit (Figure. 2c), which possesses the typical characteristic of Michaelis dynamic curve. And further the v_{max} and K_m are gotten by Lineweaver-Burk method and they are 0.0519 mmol·4min^{-1} and 13.7300 mmol·L^{-1}, respectively (Figure. 2d).

3.2. Suitability of CCS as biocatalyst

To rapidly increase dissolved oxygen in aquaculture water under emergency situation, it is imagined that SHS is taken as oxygen-released agent for its stability, neutrality and harmlessness in water, and CCS from potato starch process is taken as a biocatalyst to catalyze SHS decomposition to rapidly release oxygen for its high activity and inexpensive reason. It is because pure catalase is expensive and not suitable for practical application though catalase has the characteristic of high catalytic efficiency generally possessed by enzyme preparations [32]. Therefore, here CCS from potato starch process is considered to be employed in catalyzing SHS to rapidly released oxygen for that it is usually disposed as waste liquid and certainly cheap in application [33]. In terms of kinetic parameters, the v_{max} value is not big enough and also the K_m value is not small enough (Figure. 2c,d), but here the catalyst is CCS in which the total protein content is only 0.31%. It is well understood that actually the activity of pure catalase in CCS is high.



(a) (b)



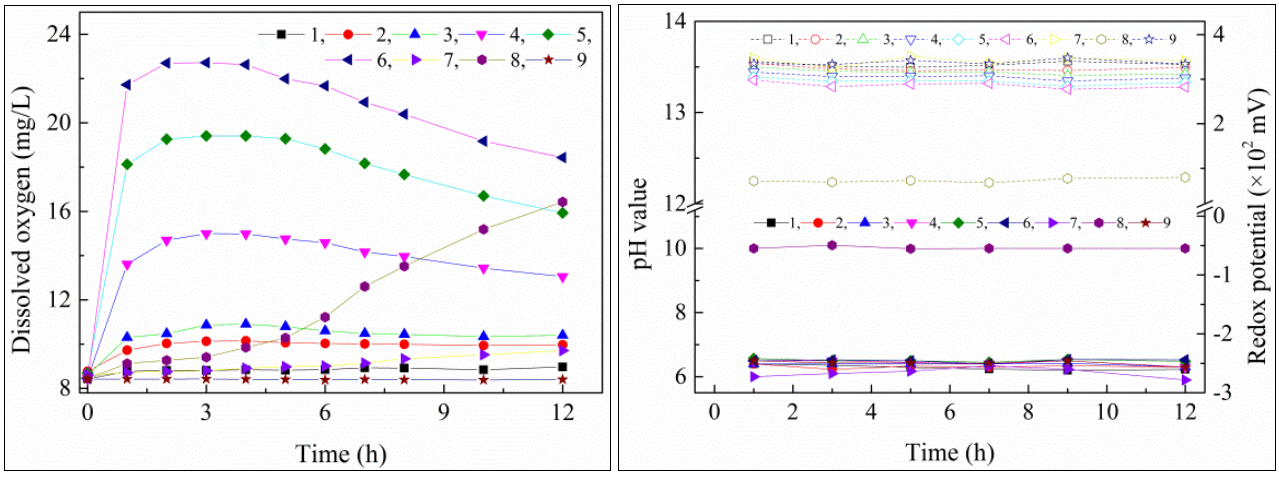
(c) (d)

Fig 2: Relative activity of crude catalase solution at different temperature (a) and pH value (b), and its kinetic curve (c) and straight line (d).

3.3. Dissolved oxygen, pH and redox potential of several systems with and without CCS

When CCS were used in catalyzing SHS, it was found that dissolved oxygen increased with volume of CCS mixed in reaction systems and achieved at 21.7 mg·L^{-1} after 1 h if CCS was 6 mL (Figure. 3a). Based on the saturated solubility of oxygen being about 8.7 mg·L^{-1} in water at measurement temperature 22 ℃ [34], it is known that dissolved oxygen in nine systems are closed to saturation at the beginning. After 1 h, dissolved oxygen in the first eight systems all were supersaturated state, namely there were oxygen generated for decomposition of peroxides in them besides of the ninth system in which no peroxide existed. But the oxygen-released rates of them were markedly different though the equivalent concentrations of HP included in them were same, being 0.043g·L^{-1} < 0.050 g·L^{-1}, safe generally for aquatic animals as well as planktons [35-37]. For example the system with SHS and 2 mL CCS, it has a oxygen-released rate 5.16 mg·L^{-1}·h^{-1}, which is 7.6 folds of the rate 0.68 mg·L^{-1}·h^{-1} possessed by the system with SP commonly taken as a marketed oxygen-released agent. Before 5 h, all the systems with CCS had dissolved oxygen concentrations higher than the system only with SHS, HP or SP. Especially, the systems with CCS over 2 mL had more much dissolved oxygen for rapidly oxygen-generated rate in them so as to a large number of oxygen bubbles were found releasing from them, but decreased in dissolved oxygen concentration after 5 h, which may be because, on the one hand, oxygen is continuously released from water to atmosphere for super saturation, on the other hand, CCS activity has some decrease and also SHS leaves little.

Differently, the system with only SP had relatively high dissolved oxygen concentration only after 6 h (Figure. 3a), but SP always caused solution with remarkably high pH values around 10 and very low redox potentials 70 mV or so (Figure. 3b), which is not conducive to the aquatic animals, especially those in partially alkaline aquaculture water [21, 22]. By comparison, CCS and SHS as well as HP did not give large changes in pH values and redox potentials of solutions.



(a) (b)

Fig 3: Dissolved oxygen of nine systems containing 500 mL distilled water and different additives: 0.453 g·L^{-1} SHS + 0 mL CCS (1), 0.453 g·L^{-1} SHS + 0.5 mL CCS (2), 0.453 g·L^{-1} SHS + 1 mL CCS (3), 0.453 g·L^{-1} SHS + 2 mL CCS (4), 0.453 g·L^{-1} SHS + 4 mL CCS (5), 0.453 g·L^{-1} SHS + 6 mL CCS (6), 0.043 g·L^{-1} HP (7), 0.15 g·L^{-1} SP (8) and nothing (9) (a), and pH values and redox potentials of the nine systems (b)

3.4. Effect of re-adding catalase on dissolved oxygen concentration

To further understand the oxygen-released ability of systems with SHS and CCS, equal volume of CCS were again added in the corresponding systems after 6 h. The results showed that most of systems with CCS added behaved some increase

once more in dissolved oxygen concentration (Figure. 4), comparing with the similar systems without CCS supplemented (Figure. 3a). Among them, the systems with CCS added by 1 mL, 2 mL and 4 mL gave more distinct increase in dissolved oxygen concentration, which means that the three systems still have SHS existed and the CCS added previously has decrease in activity along with time prolonging. The system with 0.5 mL CCS did not exhibit big increase in dissolved oxygen concentration after 0.5 mL CCS re-added, which should be ascribe to total CCS being insufficient in catalyzing SHS decomposition in the system. The system with 6 mL CCS also had not sufficiently differentiated change even if there is another 6 mL CCS added, which is concluded that SHS exists little for rapidly oxygen-released rate in the system. On the whole, the systems with SHS and 2-4 mL CCS display continually high oxygen-released rate and can meet with the urgent needs of aquaculture water body under drastic conditions, for instance the demand to increase dissolved oxygen for 4 or up to 12 h [4].

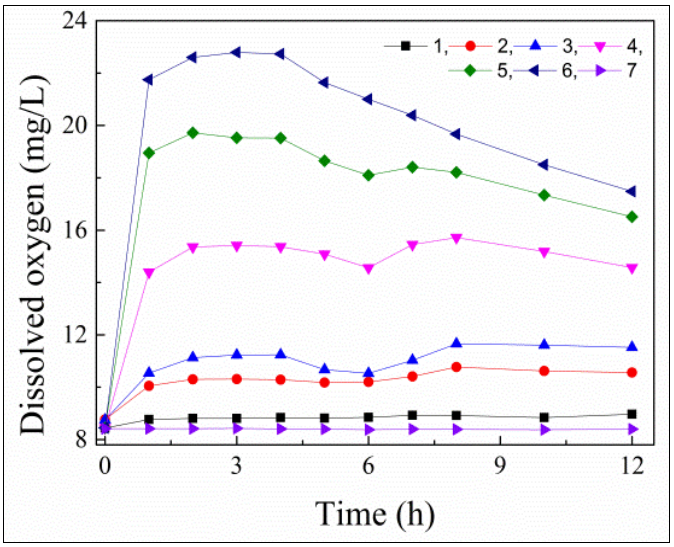
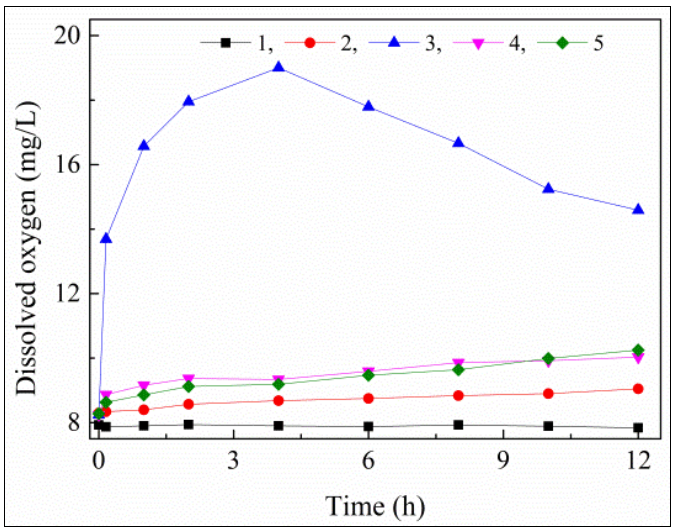


Fig 4: Dissolved oxygen of seven systems containing 500 mL distilled water and different additives: 0.453 g·L^{-1} SHS + 0 mL CCS (1), 0.453 g·L^{-1} SHS + 0.5 mL CCS (2), 0.453 g·L^{-1} SHS + 1 mL CCS (3), 0.453 g·L^{-1} SHS + 2 mL CCS (4), 0.453 g·L^{-1} SHS + 4 mL CCS (5), 0.453 g·L^{-1} SHS + 6 mL CCS (6) and nothing (7), among which the systems (2-6) again added equal volume of CCS after 6 h.

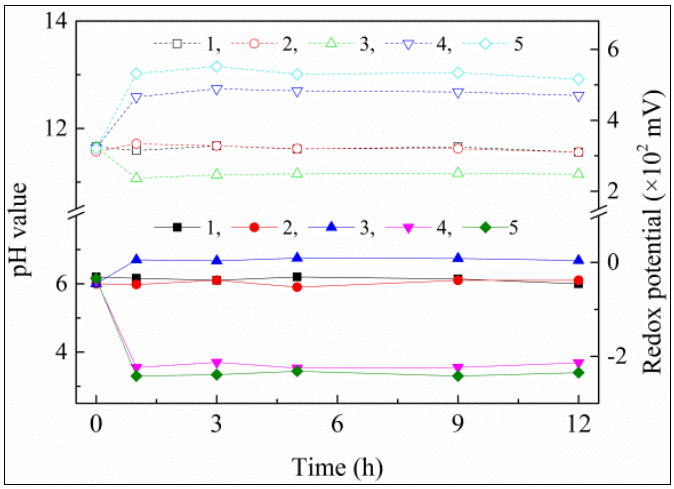
3.5. Comparing CCS with Fe^{2+}/Fe^{3+} in catalyzing SHS releasing oxygen

To refer the dosages of $FeSO_4·7H_2O$ as Fenton reaction catalyst and HP as substrate [25], the systems of Fe^{2+}/Fe^{3+} catalyzing SHS were designed and compared with the system with SHS and CCS (Figure. 5a). It was found that SHS catalyzed by 3 mL CCS brought plenty of dissolved oxygen before 4 h though thereafter dissolved oxygen decreased gradually for SHS consumed and oxygen ceaselessly escaping into atmosphere. On the other hand, Fe^{2+} or Fe^{3+} accelerated SHS decomposition less, being slightly higher than SHS self-decomposition reaction. For the 1st h, the increased oxygen in systems with CCS, Fe^{2+} and Fe^{3+} were 8.33, 0.92 and 0.58 mg·L^{-1}·h^{-1}, respectively. Comparing with Fe^{2+} and Fe^{3+} salts, here CCS containing total protein only one fifth of iron mass displays great catalysis in increasing dissolved oxygen.

The pH values of systems with SHS and SHS + CCS were about 6 and 7 similar to pH of distilled water measured, but Fe^{2+} and Fe^{3+} caused solutions down to pH 3.5 (Figure. 5b), which also is harmful to aquatic animals [38]. Moreover, CCS causes redox potential down little, relative to 300 mV redox potential of distilled water, but Fe^{2+} and Fe^{3+} caused potential values of solutions up to 480 mV and 520 mV, due to high positive charges of them. Obviously CCS is more effective in promoting SHS decomposition to release oxygen, and also better in stabilizing pH and maintaining redox potential of water body.



(a)



(b)

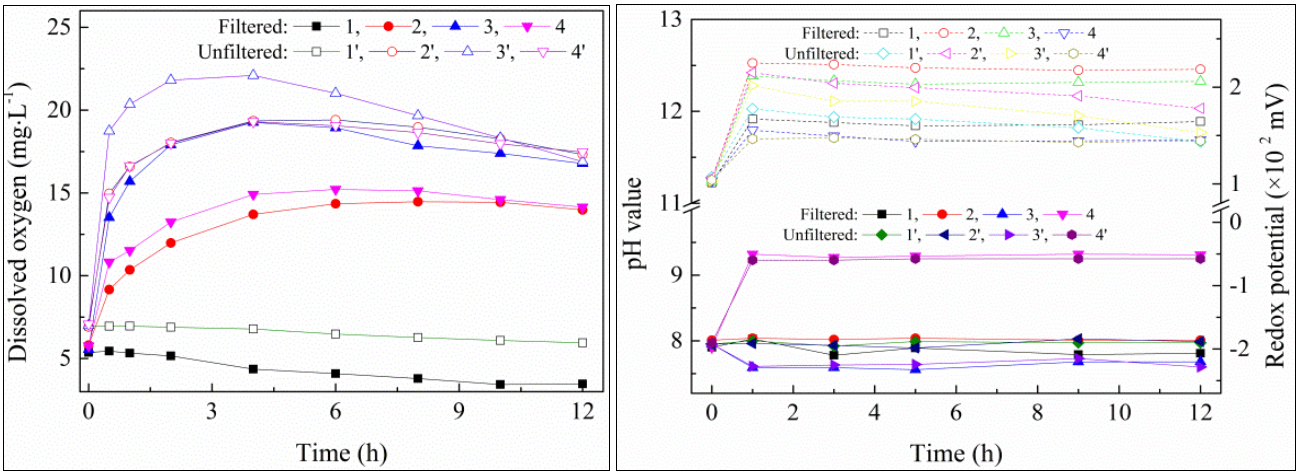
Fig 5: Dissolved oxygen of five systems containing 500 mL distilled water and different additives: nothing (1), 0.453 g·L^{-1} SHS (2), 0.453 g·L^{-1} SHS + 3 mL CCS (3), 0.453 g·L^{-1} SHS + 8 mL 2.78 g·L^{-1} $FeSO_4·7H_2O$ (4), and 0.453 g·L^{-1} SHS + 8 mL 2.78 g·L^{-1} $FeCl_3·6H_2O$ (5) (a), and pH values and redox potentials of the five systems (b).

3.6. Increasing dissolved oxygen in aquaculture water

Based the aforesaid works, releasing dissolved oxygen by CCS-promoted decomposition of SHS was carried out in aquaculture water and compared with the marketed SP (Figure. 6). In filtered aquaculture water, SHS catalyzed by CCS still was fine in increasing dissolved oxygen, being similar to that in distilled water (Figure. 5a). Both of SP and SHS had some increase in increasing dissolved oxygen, relative to that in distilled water (Figure. 3a). In unfiltered aquaculture water (Figure. 6a), SHS catalyzed by CCS showed more powerful oxygen-released ability before 6 h but, since then, weakened, especially after 8 h. SP and SHS also

displayed an oxygen-released intensity as SHS catalyzed by CCS in filtered waters. This should be derived from the effect of some substances existed in unfiltered water in which there are much constituents such as sediment particles, planktons, algae, microbial, organic and inorganic substances, etc. [39] Filtration by 0.45 μm membrane can separate most of sediment particles, planktons, microorganisms. Among them, sediment particles commonly have catalysis on peroxides [40], especially in alkaline water [41] (Figure. 6b). So the decomposition of SHS and SP is strengthened in filtered and unfiltered aquaculture water.

As to pH and redox potential of aquaculture water (Figure. 6b), CCS and SHS did not give large effect on them, which were kept between pH 7-8 and located in 150-230 mV, respectively. Differently SP resulted in pH over 9 and redox potential in-between 120-150 mV no mater it was in either filtered or unfiltered water, which all were unfavourable for aquatic animals.



(a) (b)

Fig 6: Dissolved oxygen of eight systems containing 500 mL filtered aquaculture water with nothing (1), 0.453 g·L^{-1} SHS (2), 0.453 g·L^{-1} SHS + 3 mL CCS (3), and 0.15 g·L^{-1} SP (4), or 500 mL unfiltered aquaculture water with nothing (1'), 0.453 g·L^{-1} SHS (2'), 0.453 g·L^{-1} SHS + 3 mL CCS (3'), and 0.15 g·L^{-1} SP (4') (a), and pH values and redox potentials of the eight systems (b).

4. Conclusions

To rapidly increase dissolved oxygen in aquaculture water under emergency situation, it is imagined that SHS is taken as oxygen-releasing agent for its stability and neutral characteristic in water, and CCS from potato starch processing process is employed as a biocatalyst to catalyze SHS decomposition to rapidly release oxygen for its high activity and inexpensive reason. CCS contains 0.31% of total protein and has stable activity in 22-28 ℃ and pH 6-9 commonly fitted for aquaculture environment. If the equivalent concentration of hydrogen peroxide included in various oxygen-releasing agents in 500 mL water is set at 0.043 g·L^{-1} lower than 0.050 g·L^{-1} safe for aquatic animals and planktons, SHS as oxygen-releasing agent catalyzed by CCS more than 2 mL can rapidly increase dissolved oxygen by over 5.16 mg·L^{-1}·h^{-1}, which is 7.6 folds of the rate 0.68 mg·L^{-1}·h^{-1} possessed by the marketed oxygen-releasing agent SP. And also the former has little impact on pH and redox potential, and the latter results in pH up to 10 and redox potential down to 70 mV, being harmful to aquatic animals. Even if there is some decrease in dissolved oxygen concentration in the system with SHS and CCS after 6 h, the re-added CCS can give increase in dissolved oxygen concentration, which shows that SHS still exists, namely the increase of dissolved oxygen is not only rapid but also sustained. Comparing with Fe^{2+} and Fe^{3+} salts, CCS containing total protein only one fifth of iron mass displays great advantages in increasing dissolved oxygen, stabilizing pH and maintaining redox potential, and two kinds of iron salts are weak in increasing dissolved oxygen and also cause pH down to 3.5 and redox potential up to more than 480 mV. In aquaculture water, SHS catalyzed by CCS still is excellent in increasing dissolved oxygen, especially in unfiltered aquaculture water before 6 h, and also has little impact on pH and redox potential, unlike SP.

All in all, the dissolved oxygen rapidly increased by CCS promoted decomposition of SHS is effective and inexpensive in increasing dissolved oxygen in aquaculture water. However, the researches still need carried out in the future, for example, enriching catalase from CCS into solid enzyme by convenient methods and preparing tablets with SHS for better use in aquaculture.

5. Acknowledgements

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