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Supporting information

Reaction chemistry and engineering

Merging of chemical reaction with microbial metabolism via inverse phase transfer catalysis for efficient production of red *Monascus* pigments

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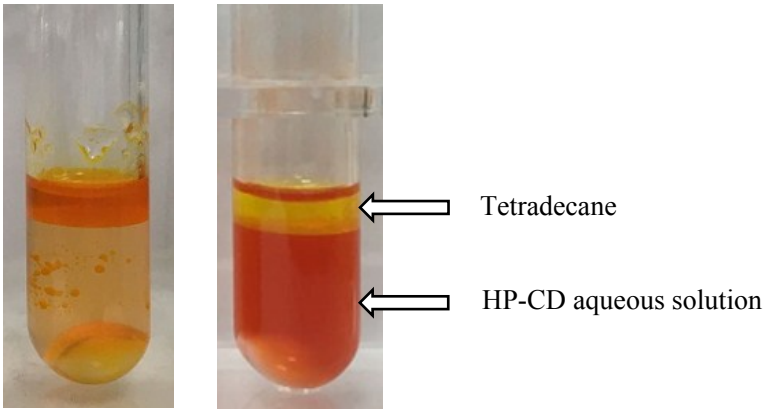
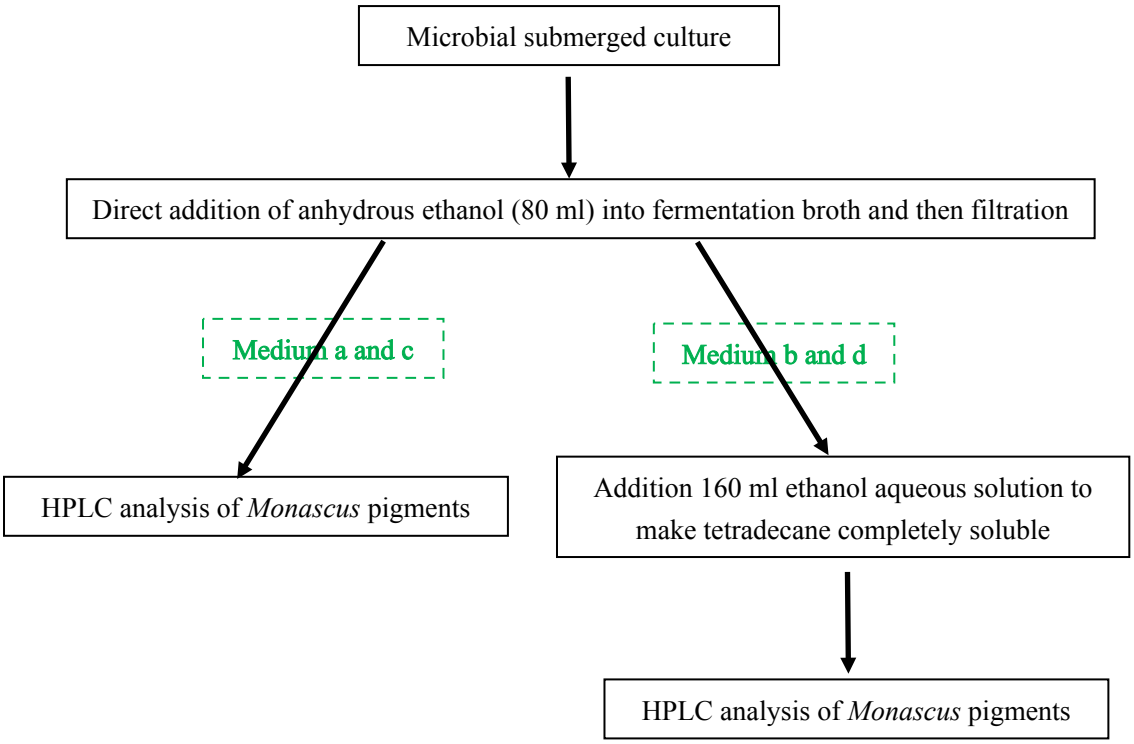
S1 Sample preparation for quantitative analysis of *Monascus* pigments

During inverse phase transfer catalysis of chemical modification of OMPs into RMPs-MSG (Fig.3 and Fig.4), tetradecane separated from the HP-CD aqueous solution instinctively and formed a water-tetradecane two-phase system. Fig.S1A showed the mixing of 2 ml HP-CD aqueous solution with 0.5 ml tetradecane, where OMPs was 0.2 g/l, MSG 6 g/l, and HP-CD 20 g/l concentration based on the volume of HP-CD aqueous solution. Before the chemical reaction, OMPs were partitioned into the tetradecane phase. After reaction for 12 hours, the highly water-soluble RMPs-MSG were partitioned into the HP-CD aqueous solution phase, while OMP still stayed in the tetradecane phase. Thus, RMPs-MSG concentration was determined directly by properly dilution of the aqueous solution phase with ethanol aqueous solution (70% ethanol, pH=2). A similar procedure was applied for determination of RMPs-MSG concentration during microbial fermentation of RMPs-MSG (Fig.6 and Fig.7A).

However, for determination of water-insoluble OMPs in the microbial fermentation broth by HPLC analysis was another case. Under this condition, the solvent ethanol was added directly to the whole fermentation broth before filtration (Fig.S1B). After filtration, filtrate was an (HP-CD) aqueous solution (medium a and medium c in table 1), which was applied directly for HPLC (high performance liquid chromatography) analysis of OMPs, RMPs, and YMPs concentration after dilution properly. While the filtrate was a water-tetradecane two-phase system (medium b and medium d in table 1) where OMPs were partitioned into the tetradecane phase (Fig.S1A). The volume ratio of (HP-CD) aqueous solution to tetradecane was 40 to 1.5 (Fig.5) or 1 (Fig.7B). In this case, another 160 ml of ethanol aqueous solution (70% ethanol, pH=2) was furtherly added into the ethanol aqueous solution to make sure the tetradecane completely soluble. The whole ethanol aqueous solution was utilized to HPLC analysis of OMPs, RMPs, and YMPs concentration.



A: Determination of RMPs-MSG concentration during inverse phase transfer catalysis



B: Procedure for sample preparation during microbial culture in different media

Fig.S1 Sample preparation for quantitative analysis of *Monascus* pigments

S2 Identification and quantification of *Monascus* pigments

After submerged culture of *M. aurantiacus* in aqueous solution, most of OMPs were attached on the mycelia as crystals (Lv et al., 2018). OMPs or YMPs were isolated from fermentation broth and then dissolved in an ethanol aqueous solution (70 %, V/V, pH=2). The ethanol aqueous solution was utilized for UPLC-MS analysis as well as preparation of standard concentration curve.

The molecular formula of *Monascus* pigments was identified by UPLC-MS analysis. UPLC-MS analysis was performed using ACQUITY & Q-TOF MS Premier (Waters, USA; column: ACQUITY UPLC BEH column, 2.1×100mm, 1.7μm) in positive ionization modes. The column temperature was maintained at 50 °C and the gradient eluting program was started from 10 % acetonitrile and 90 % water, and changed to 100 % acetonitrile within 5 min, then maintained 100 % acetonitrile within 5 min, and changed to 10 % acetonitrile within 0.5 min, and at last by equilibration at 10 % acetonitrile for 2.5 min at a flow rate of 0.4 ml/min. The sample injection volume was 5 μl. MS analysis was carried out via Masslynx 4.1 software (Waters MS Technologies, Manchester, UK). The parameters in the source were set as follows: capillary voltage 3.0 kV,

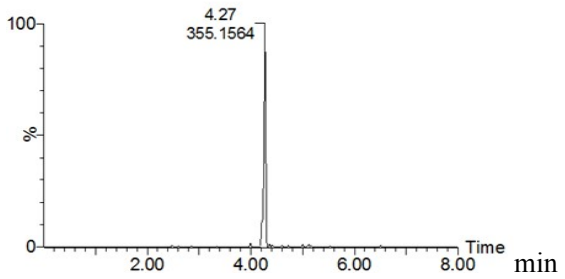
sample cone 35.0 V, source temperature 100 °C, desolvation temperature 350 °C, collision energy 4.0 eV, cone gas flow 50 l/h, and desolvation gas flow 600 l/h. Data were collected in centroid mode from mass-to-charge ratio (m/z) 50 to 1000 at scan time of 0.25 s with an interval of 30 s.

A certain amount of solid pigments were dissolved in an ethanol aqueous solution (70%, V/V, pH=2). The pigment solution was diluted properly with the ethanol aqueous solution to prepare a series of samples with different pigment concentration. Those samples was utilized for preparation of standard concentration curve of pigments by HPLC analysis. HPLC was performed using Shimadzu LC-20AT system with a photodiode array (PDA) detector. An InertSustain C18 column, 4.6×250 mm id., 5 μm (GL Sciences Inc., Japan), was used as analytical column. Running with acetonitrile/water (70:30, V/V) as mobile phase with a flow rate of 1ml/min, pigment with distinguish retention time was detected at wavelength 500 nm otherwise specified.

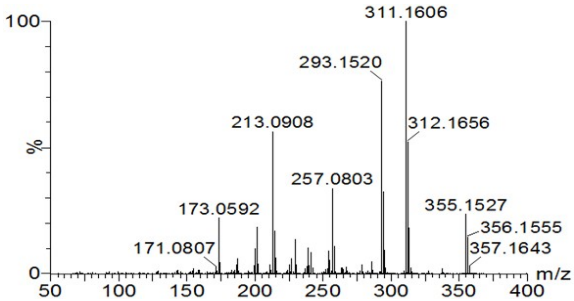
Lv, F., Liu, L., Huang, Y., Zhang, X., Wang, Z, 2018. Production of *Monascus* pigments as extracellular crystals by cell suspension culture. Appl. Microbiol. Biotechnol. 102: 677-687

OMPs

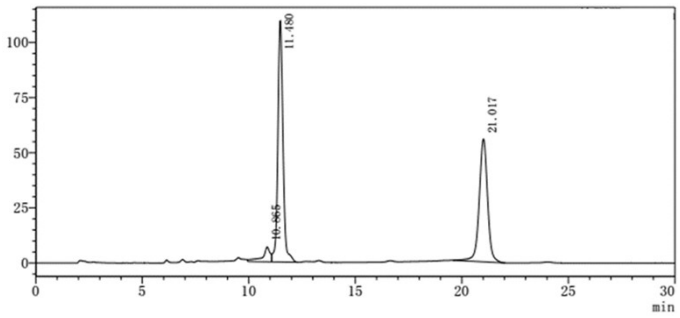
As shown in scheme 2, OMPs includes rubropunctatin (R=C_5H_{11}) and monascorubrin (R=C_7H_{15}). The basic data of rubropunctatin by MS analysis and the standard concentration curve of mixed OMPs obtained from fermentation broth were presented in Fig.S2.



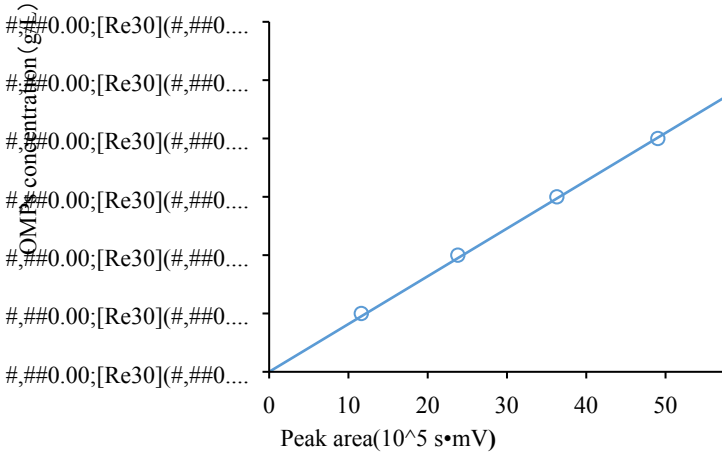
A: Extracted ion chromatogram of rubropunctatin



B: MS/MS fragmentation pattern of rubropunctatin



C: Rubropunctatin and monascorubrin with retention time of 11.5 and 21min, respectively

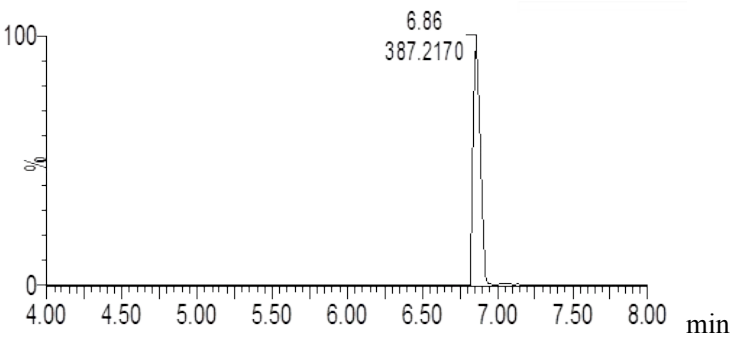


D: OMP standard curve detected at 500nm

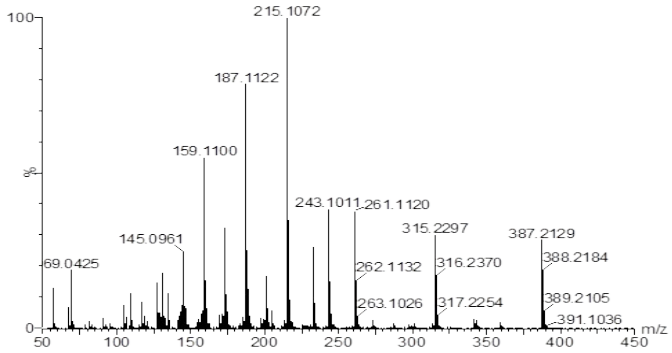
Fig.S2 Basic data of OMPs

YMPs

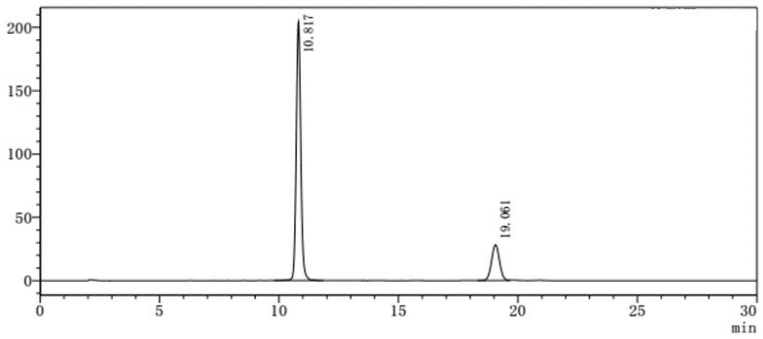
As shown in scheme 2, YMPs includes monascin (R=C_5H_{11}) and ankaflavin (R=C_7H_{15}). The basic data of ankaflavin by MS analysis were presented in Fig.S3.



A: Extracted ion chromatogram of ankaflavin



B: MS/MS fragmentation pattern of ankaflavin

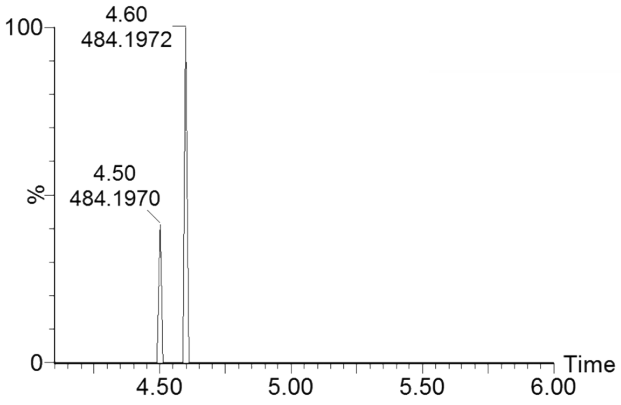


C: Monascin and ankaflavin with retention time of 10.8 and 19.0 min, respectively

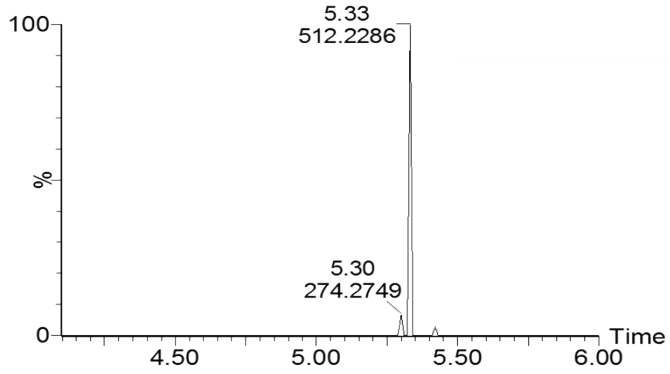
Fig.S3 Basic data of YMPs

RMPs-MSG

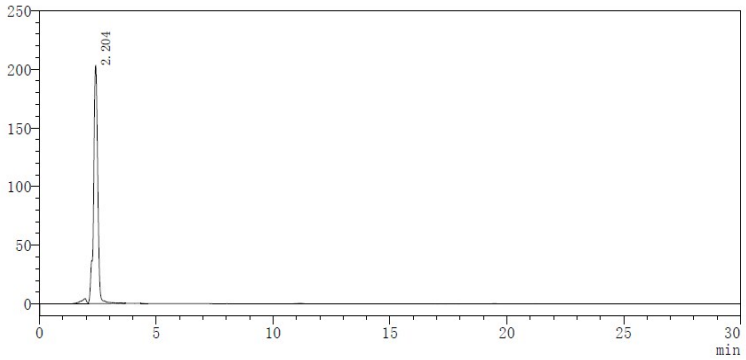
As shown in scheme 3, YMPs-MSG includes two molecules with R=C_5H_{11} and R=C_7H_{15}. The basic data by MS analysis and the standard concentration curve of mixed YMPs obtained from fermentation broth were presented in Fig.S4.



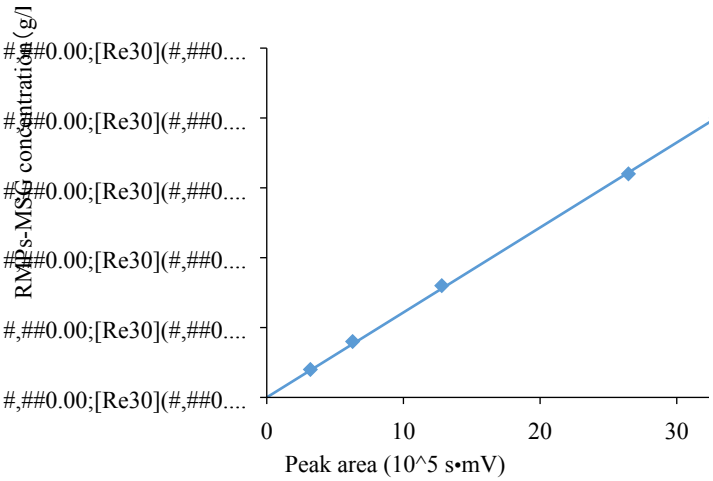
A: Extracted ion chromatogram of OMP-MSG with R=C_5H_{11}



B: Extracted ion chromatogram of OMP-MSG with R=C_7H_{15}



C: RMPs-MSG only with a single retention time 2.2 min



D: RMPs-MSG standard curve detected at 500nm

Fig.S4 Basic data of RMPs-MSG

S3 Analysis of residual glucose concentration

After submerged culture, the whole fermentation broth was subjected to filtration. The aqueous solution phase of the fermentation broth was utilized for residual glucose concentration analysis by a standard 3, 5-dinitrosalicylic acid method (DNS, Miller, 1959) with a spectrophotometer.

The basic analysis procedure was as follows: First, an analysis reagent was prepared: 6.3 g of 3, 5-dinitrosalicylic acid was dissolved in 262 ml of 2M NaOH solution. 185 g of potassium sodium tartrate tetrahydrate was dissolved in 500 ml of hot water (about 45 ^{o}C). Both of them were combined and 5 g of anhydrous sodium sulfite and 5 g of phenol were added consecutively. Then the solution was diluted with distilled water to 1000 ml. Second, 2 ml of the diluted sample solution was mixed with 1.5 ml of the analysis reagent, which was reacted in a boiling water bath for 5 min. The reaction solution was cooled to room temperature and diluted to 25 ml with distilled water. Finally, the diluted solution was detected at a wavelength 540 nm with a spectrophotometer and the same aqueous solution without glucose followed the same procedure was used as a control. The standard glucose concentration vs its absorbance at 540 nm was presented in Fig.S5.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31 (3): 426-428

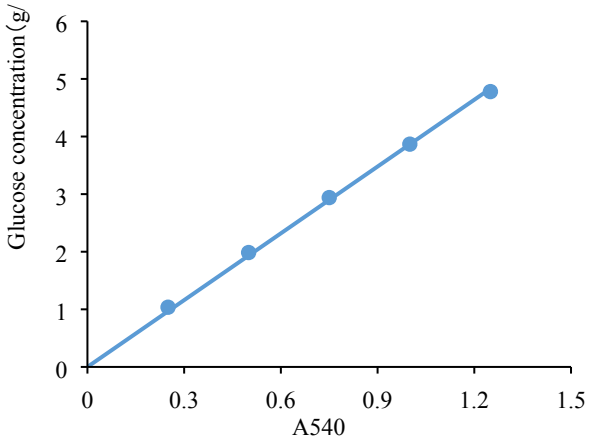


Fig.S5 Standard concentration of glucose aqueous solution

S4 Selection of organic solvents for inverse phase transfer catalysis

Chemical reactions between OMP and MSG (Scheme 3) was carried out in 2 ml HP-CD aqueous solution as control, where OMP concentration was 0.2 g/l and HP-CD concentration was 20 g/l . Other chemical reaction was carried out in the 2 ml HP-CD aqueous solution by adding 0.5 ml different organic solvents. The chemical reaction was initiated by stirring the mixture 200 rpm at room temperature (25-28 ℃). After reaction 12 hours, two milliliters of ethanol aqueous solution (pH =2 and the volume ratio of ethanol to aqueous solution =7:3) was added to stop the chemical reaction. The product RMPs-MSG concentration of the reaction was determined by HPLC analysis. The results were presented in Fig.S6, where tetradecane exhibited as a good competitor.

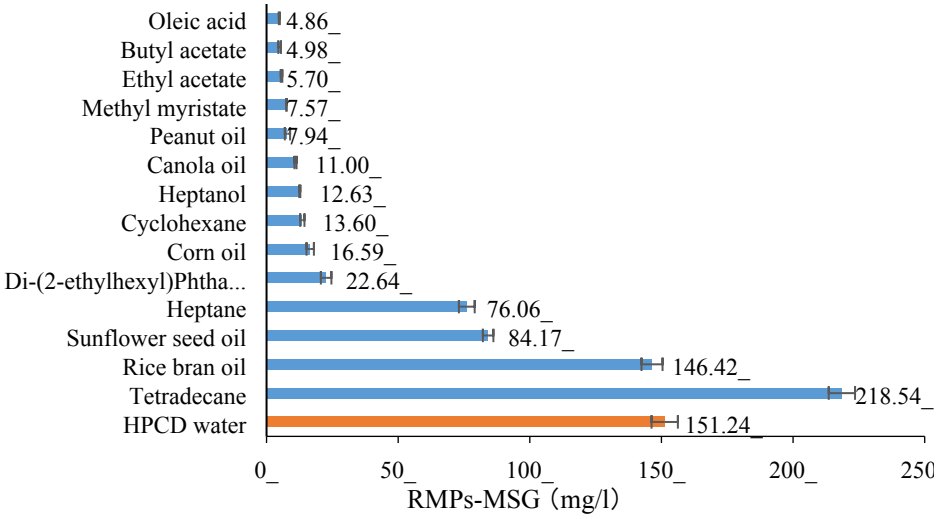


Fig.S6 Selection of organic solvents for inverse phase transfer catalysis

S5 Effect of pH on stability of OMPs

One milliliter of organic solvent (0.2 g/l OMPs in chloroform) was added into each tube and let the organic solvent evaporate under 40 ℃ condition. Two milliliters of ethanol aqueous solution (the volume ratio of ethanol to aqueous solution =7:3) with different pH (such as 2, 4, 6, 7) was added into each tube. Each sample was placed in a constant temperature incubator for test (30 ℃, 200 rpm). 4 ml ethanol aqueous solution (pH =2 and the volume ratio of ethanol to aqueous solution =7:3) was added to stop the chemical degradation every 24 hours. OMP concentration in the aqueous phase was estimated by visible spectrum (470nm) and represented as absorbance unit. As shown in Fig.S7, OMP kept stabile at pH=2 while degraded significantly at pH=7.

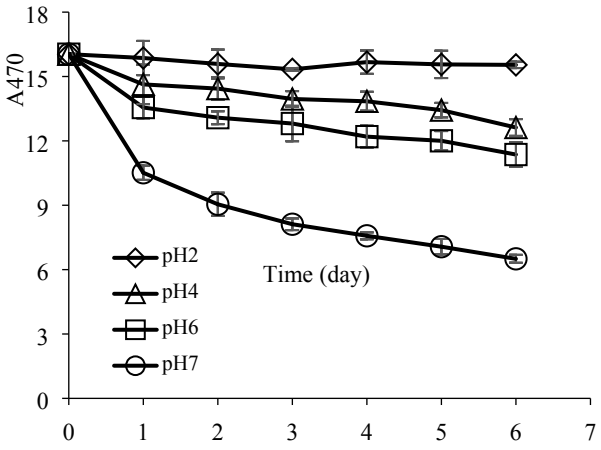


Fig.S7 Stability of OMPs at different pH values