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Biodegradability of Pollutants from Cooking Fumes

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Abstract Experiments were carried out to study the biodegradability of pollutants from cooking fumes by active sludge domestication. The result indicated that temperature was the most important factor affecting biodegradation, and under the optimum conditions, the concentration of the pollutants from cooking fumes decreased from 56.9 to 0.78 mg/L in 28 h, the specific degradation rate reached 0.15 mg (oil)/mg (biomass) and the specific degradation speed approached 0.13 mg (oil)/h/mg (biomass) when the concentration of the pollutants was higher than 352 mg/L. The dominant strains consisted of bacteria, filiform fungi and yeast fungi, and yeast fungi were identified as *Rhodosporidium toruloides*.

Keywords Cooking fume · Optimal conditions · Specific degradation speed · Dominant strains

Cooking fumes are the pyrolysate of fats and fried food. The reaction products include the carbohydrate gas, proteins, and amino acids during or after cooking (Zhang 2001). The chemical components of cooking fumes vary with the kinds of food, spices, cooking oil types and cooking methods (Liu 1999). Cooking oil usually refers to vegetable oil and animal oil. Vegetable oil includes bean oil, colza oil and peanut oil, etc. The main components of vegetable oil are unsaturated fats such as flax acid and suboleic acid, etc. Animal oil usually refers to lard, whose main component is saturated fatty acid glyceride. Cooking fumes not only contain a large amount of oil aerosol, but also consist of a great number of volatile organic compounds (VOCs). Cooking fume can cause great harm to humans, for example, coughing, immunity toxicity, mutation, etc. (Siegmann and Sattler 1996). At present, the main methods of treating cooking fumes are those utilizing physics and physical chemistry (Su et al. 2000), however, the effective time period of these methods is very short due to the special viscosity of cooking fumes, and the contamination is difficult to remove. Biological method may provide a good way to resolve this problem, since many studies on the treatment of fatty wastewater by biodegradation have been reported (Kulowiec 1979; Tano-Debrah et al. 1999; Ratledge 1992). The acclimationed dominant strains in sludge work on the targeted pollutant playing a key role in the process of biodegradation of fats and oils (Koh et al. 1983; Shirai et al. 1998). Activated sludge acclimation and culture is a simple and effective method of obtaining dominant strains (Anon 1994a, b). Recently, (Sun et al. 2002) reported that a dominant strain of *Pseudomonas Putida*, which was obtained by using the cultivation method in the gas phase, shows a high degradation effectiveness (as high as an average of 96%) of toluene in waste gas. However, to the best of our knowledge, a study on the dominant strains' degradation of pollutants from cooking fumes via activated sludge acclimation and screening has not been reported. Therefore, in this paper, attempts were made to acclimate and identify the dominant microorganisms capable of decomposing pollutants from cooking fumes. Morphological analysis and biological of identification, advanced technology of a DNA sequencing test were used to identify the special strains.

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Materials and Methods

In this study, the waste oil in kitchen exhaust system was heated as cooking fumes, and the bacteria culture bouillon agar (NA) and fungus culture medium were offered by Guangdong Huangkai Microbe Technology Limited Company, the bacterial conservation medium was prepared using the standard medium and agar 20.0 g/L and was put into different sterile tubes to form slopes. The mould conservation medium (PDA) was composed of: potato, 200.0 g/L; cane sugar, 20.0 g/L; agar, 20.0 g/L. The pH was adjusted to 6.0–7.0, then the sterilized medium was encased in test tubes to form slopes. The yeast conservation medium (YPD) was composed of malt extract agar medium and 20.0 g/L agar, then the medium was made into flats. All the mediums were sterilized for 30 min under steam at 121°C.

The activated sludge taken from the aeration tank of the sewage treatment plant was aerated using pump in order to maintain activity and was used as the inoculum of the acclimation microorganism for treatment of the cooking fumes, and the activated sludge acclimation was carried out in a rockered flask as shown in Fig. 1. The acclimation process can be described as follows: First, 250 mL supernatant of activated sludge was placed in a 1000 mL rockered flask, then 5 ml of waste grease (a mixture of waste oil and blended cooking oil at a ratio of 3:1, respectively) and 5 mL inorganic salt medium were added. The mixture sludge/grease/salt medium (sgsm) was diluted with sterile distilled water to 500 mL and incubated at 30°C for 6 days. Three times a day, waste oil and cooking oil were mixed and heated and the mixture fumes were pumped into the sgsm mixture using X-6500 pump for 1–2 h each time. After 6 days, the obtained culture was marked as "a" inoculum. From then on, samples of "a" inoculum (25 mL), were taken from the flask daily, and stored at 5°C for use in subsequent analysis. Then 100 mL of the "a" inoculum was taken out and placed in another flask, 10 mL grease and 5 mL inorganic salt medium were added. The "a" sgsm mixture was diluted with sterile distilled water to 500 mL and using the same operational process as before, obtained the culture marked as "b" inoculum. Finally, 100 mL of the "b" inoculum was taken out and placed in the third flask, 20 mL grease and 5 mL inorganic salt medium and sterile distilled water were added, and the same operation was repeated. The obtained culture was marked as "c" inoculum. The culture "c", obtained at the end of the supernatant of activated sludge, was described as acclimated sludge and stored at 5°C.

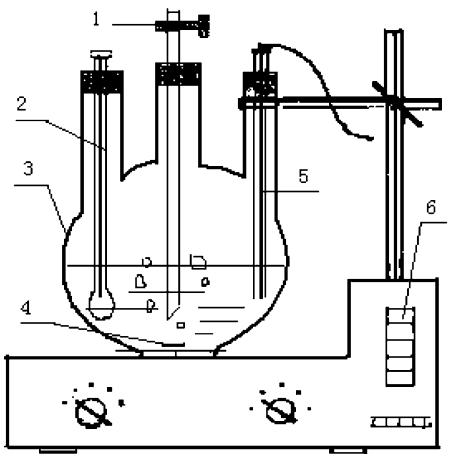

Fig. 1 Experimental equipment: 1, aeration sampling; 2, thermometer; 3, culture bottle; 4, magnetic stick; 5, sensor; 6, thermostatic magnetic stirrer

In order to identify the main microorganism species and explain the change of microorganism population before and after acclimation, the original activated sludge and cultures "c" which had been acclimated for three weeks were inoculated and cultured using a characteristics medium. Then the variability of the dominant strains before and after acclimation were further characterized by the denaturing gradient gel electroporesis (DGGE) method, and the dominant strains' pure cultures were streaked on dishes and were identified by morphological character and the DNA sequencing test.

Analytical methods of the concentration of the liquid grease: samples were extracted by petroleum ether, then were filtered by anhydrous sodium sulfite (Na_2SO_4). The absorption, at 280 nm, of the filtrate was recorded by ultraviolet spectrophotometer, and the concentration of the samples was determined by using the absorption standard curve of the standard oil. The density of the liquid microorganism population was determined by testing of the concentration of aqueous protein, since the protein was the main component of the microorganism. Generally, "protein count ×5" was used to express the active biomass in microorganism engineering. The samples were dyed by Cu^{2+} and their absorption, at 228 nm, was recorded. The absorption standard curve of the standard bovine serum albumin (ABS) samples was used to determine the concentration of the samples.

Result and Discussion

Under sterile conditions, the original activated sludge and cultures "c" were diluted, each to twice their original volumes. Then 100 μL diluted samples were placed on four different culture mediums, bacterial nutrient broth agar (NA), fungi, yeast malt extract agar and actinomycete (Chigusa et al. 1996). The process was repeated three times. Keeping the temperature of the biochemical incubator at 28–30°C, the bacterial NA cultures were incubated

for 2 days, filiform fungi cultures for 3 days, and actinomycete cultures for 5 days. The colonies were classified and counted, Table 1 shows the differences between the original activated sludge and the cultures "c" which has been acclimated for three weeks as follows (1) bacterial population density and species showed decreases of 16000 cfu/mL in the sludge to 1500 cfu/mL in "c" culture and from eight species to two. (2) Nothing changed in the species of fungi occurring before and after acclimation, both contain a species of filiform and two species of yeast. But the population of fungi increased markedly, with the population of filiform fungi increasing from 900 to 48300 cfu/mL and yeast fungi from 300 to 47500 cfu/mL. This result indicated that the fungi grew fast and showed dominance in the process of acclimation. (3) Actinomycete was not discovered either before or after acclimation. This showed that actinomycete is difficult to isolate and cultivate or may not have existed in the original activated sludge.

Before the identification, we marked the eight species of bacteria before acclimation as 1XJ-01, 1XJ-02, 1XJ-03, 1XJ-04, 1XJ-05, 1XJ-06, 1XJ-07, 1XJ-08 and the two species of bacteria after acclimation as 2XJ-01, 2XJ-02, respectively. Then all the above bacteria were dyed by Gram reaction, and the Gram – variable – bacteria were streaked on the bacterial nutrient broth agar (NA) culture medium, and incubated for 2 days at 28–30°C in the biochemical incubator. Table 2 shows the morphological characteristics of the bacteria. From Table 2, we can see that only 1XJ-04 bacterial colonies showed Gram-negative (pink), others showed Gram-positive bacteria. After acclimation, 2XJ-01 had spores, while 2XJ-02 had no spores. The morphology of 2XJ-01 is similar to that of 1XJ-01. On the solid plane culture medium, both colonies are spherical, measuring 2–3 mm in diameter and have a moist and smooth surface, they look very thin and transparent, the edges are regular and appear highly salient. The bacteria have a rod-like morphology are arranged in ones and twos. The morphology of 2XJ-02 is similar to that of 1XJ-05, both colonies have a star-like morphology, 2–3 mm in diameter, a moist and rough surface, and look very thick and semi-transparent under the microscope, their edges are also regular and the middle seems a little salient. The single one has a rod-like body and a blunt round end. According to the morphological analysis, 2XJ-01 was identified as *Burkholderia cepacia* and 2XJ-02 was *Pseudomonas aeraginosa*. Some dominant strains could not be isolated by pure culture on the bacterial nutrient broth agar (NA) medium, so the DGGE method was used to analyze the changes of the bacterial dominant strains before and after acclimation. Since all the procaryotic microbes have such 16SrDNA genetic material, the difference, based on formation in the V_3 fragment, is a labile region, the width of this region is about 200 bp and can be isolated by the DGGE method, the size of the population and the brightness of the DGGE band are a direct ratio.

Figure 2 shows the results of DGGE analysis of the bacteria in solution before and after acclimation. The band density of the solution before acclimation is higher than that of in the number of solution after acclimation, but the

Table 1 Microorganism contrast between after and before domestication

Samples	Classification							
	Bacterium		Fungi				Actinomycete	
	Tolal	Type	Filiform		Yeast		Tolal	Type
			Tolal	Type	Tolal	Type		
Before domestication	1.60×10^4	8	9.0×10^2	1	3.0×10^2	2	0	0
After domestication	0.15×10^4	2	4.83×10^4	1	4.75×10^4	2	0	0

Table 2 Characteristics of colonies on plate culture medium (NA plate, 3 days)

Marks	Color	Morphology	Size (μm)	Surface	Transparency
1XJ-01	Yellow	Circular	2	Smooth	Transparent
1XJ-02	Milk white	Near circular	2	Pucker	Opaque
1XJ-03	Milk white	Irregular	3–4	Rough	Opaque
1XJ-04	Pink	Circular	1–2	Rough	Opaque
1XJ-05	Milk white	Star-like	2	Rough	Half transparent
1XJ-06	Milk white	Circular	2–3	Smooth	Half transparent
1XJ-07	Yellow grey	Irregular	3	Rough	Opaque
1XJ-08	Milk white	Irregular	3–4	Smooth	Opaque
2XJ-01	Milk white	Circular	2–3	Smooth	Transparent
2XJ-02	Milk white	Star-like	2–3	Rough	Half transparent

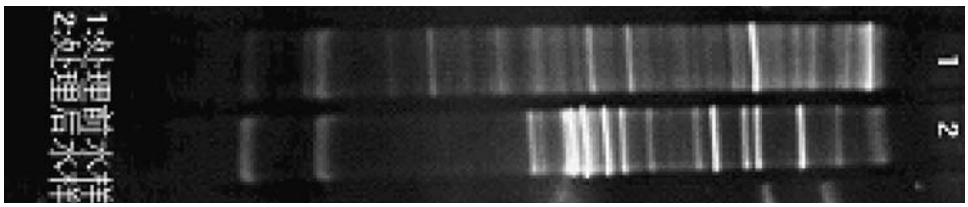

Fig. 2 The analysis result of DGGE between after and before acclimation: 1, activated sludge; 2, acclimatised activated sludge

brightness of the bands is less than the solution after acclimation, which indicates that the species of the bacteria before acclimation are greater than that of the bacteria after acclimation. The number of dominant strains increased after three weeks' acclimation. Compared to the results of pure culture (Table 1), we can see in the DGGE analysis that the number of dominant strains both before and after acclimation is greater than the results shown in pure culture, indicating that some species of bacteria can not be isolated by pure culture.

Filamentous fungi strains isolated from activated sludge (marked Z-1) and acclimatized activated sludge (marked Z-2) were streaked on fungus conservation substratum (PDA) and slopes, respectively, then they were incubated in the biochemical incubator at 28–30°C for 2 days. Figure 3a and b shows the microscope images of Z-1 and Z-2 that were cultured on fungus conservation substratum (PDA). Figure 3c shows the microscope images of Z-1 (above) and Z-2 (below) which were cultured on slopes. During the procedure of culture on PDA, the growth characteristics of filamentous fungi strains could be observed as the follows: first, white hypha appeared, then the white hypha slowly turned green from the center, and finally, green colonies were observed but the edge remained white. Based on the morphological analysis and the growth characteristics, all the filamentous fungi strains were identified as *Penicillum*.

Two acclimated and isolated yeast fungi samples "JM-01" and "JM-02" were streaked on bacterial culture bouillon agar medium plates, mould conservation medium (PDA) and malt extract agar culture medium dishes, then were incubated in the biochemical incubator at 28–30°C for 3 days. The growth process characteristics of the yeast could be observed by microscope, with the results shown in Fig. 4. Figure 4a and b shows the morphologies of JM–01 and JM-02 cultured on malt extract agar medium dishes, Fig. 4c shows the morphologies of JM-01 and JM-02 cultured on PDA medium dishes. Figure 4d shows the morphology of the single strain of JM-01 cultured on NA, Fig. 4e and f shows the whole microscope morphologies of JM-01 and JM-02 strains on NA medium. The morphology of JM-01 on NA is circular, shows a smooth surface measuring 2–3 mm in diameter, while on malt extract agar medium it is nearly circular, has burred edges and measuring 3 mm in diameter. All the JM-01 samples show white in the growth incipience, turning red when old. The numbers of strain JM-02 are lower than JM-01. JM-02 strains on the NA medium show circular morphology, milk-white color and measuring 3–4 mm in diameter. On malt extract agar culture medium, the morphologies of JM-02 are similar to JM-01, and the DNA sequencing of PCR product shows that the 18S-ITS of JM-01 is very close to *Rhodosporidium toruloides* (Identities = 99%), so the dominant strain JM-01 was identified as *Rhodosporidium toruloides*. From the identified results, we can see that the "c" inoculum solution which has been acclimated for 21 days still belongs to a microbe colony, including many strains of bacteria and fungi. Liquid cooking fumes were degraded by the collaboration of these dominant strains. Not all the dominant strains could be identified completely due to the limited experimental conditions. The dominant strains were identified by morphological analysis and molecular biology to be *B. cepacia*, *P. aeraginosa, Penicillum* and *Rhodosporidium toruloides*.

Temperature, pH value, dissolution of oxygen, the concentration of mineral solution, added nitrogen source, etc. often are the main effective factors in the

Fig. 3 The characteristics of colonies about filamentous fungi before (marked Z-1) (**a**) and after (marked Z-2) (**b**) acclimation were cultivated for pure on PDA plate; **c** the characteristics of colonies about filamentous fungi before and after acclimation (marked Z-1 and Z-2) were cultivated for pure on PDA bevel

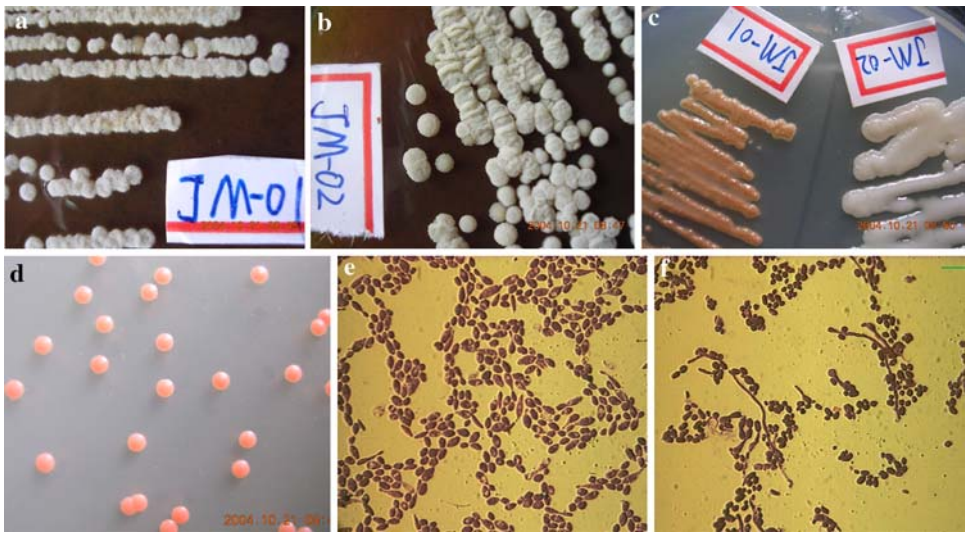

Fig. 4 Yeast contrast between JM-01 and JM-02: the characteristics of colonies about JM-01 (**a**) and JM-02 (**b**) after acclimation were cultivated for pure on NA plate; **c** the characteristics of colonies about JM-01 and JM-02 after acclimation were cultivated for pure on PDA plate; **d** the microscope images of odd colony about JM-01 after acclimation were cultivated for pure on NA plate; the microscope images of colonies about JM-01 (**e**) and JM-02 (**f**)

biodegradation (Chen et al. 2002). In our experiment, dissolution of oxygen was determined by stirring velocity, while the concentration of mineral solution was determined by the volume of nutrition solution. An added nitrogen source came from the mass of ammonium phosphate [$(NH4)_2HPO_4$]. In order to find the optimal conditions for the biodegradation of cooking fumes, an orthogonal experiment was designed based on five factors (temperature, pH value, stirring velocity, the concentration of mineral solution and added nitrogen source) and four levels. The factors and the levels are shown in Table 3, stirring velocity refers to the rotating speed of the magnetic stick, nitrogen quantum refers to the mass of $(NH_4)_2HPO_4$ added once, the quantum mineral solution refers to the volume of nutrition solution added once. The experiment condition were controlled by level factors as shown in Table 2, the level experiment was based on the method 1.2.3 for microbe degradation of cooking fumes for 2 days, menstruating the concentration of cooking fumes and microbes before and after degradation, then menstruating the specific biodegradation rate. The results and the level factors are shown in Table 4, average degradation rate of every factor was obtained by the arithmetical average method and expressed as k_{ij} (i: factor A, B, C, D, E, j: level 1, level 2, level 3, level 4). The optimal level can be obtained by comparing the value of k_{ij} under the same factor, R_i (i: factor A, B, C, D, E) showing the extremum between the maximum and the minimum of k_{ij}, the bigger k_{ij}, and the greater influence of the factor. As for factor A (stirring velocity), Table 2 shows that the optimization was at approximately 120 rpm where $k_{A3} = 0.0303 > k_{A1} = 0.0288 > k_{A4} = 0.0278 > k_{A2} = 0.024$, and the optimization of nitrogen quantum, added mineral solution quantum, pH value and temperature was 4 g, 50 mL, 7 and 37°C, respectively. From Table 4, it can see that $R_E = 0.0108 > R_B = 0.0106 > R_D = 0.0096 > R_A = 0.0063 > R_C = 0.0054$, which shows that the greatest effective factor was temperature, followed by the added nitrogen source, pH value, stirring velocity and the added mineral solution quantum, in that order.

Table 3 Level table of factors in orthogonal experiment

Factor	Level 1	Level 2	Level 3	Level 4
A: stirring velocity (r/min)	40	80	120	240
B: nitrogen (g)	1.0	4.0	5.0	10.0
C: mineral solution (mL)	0.5	10	50	
D: pH	3.0	6.0	7.0	10.0
E: temperature (°C)	18	25	30	37

Using the acclimatized strains obtained in this way, the degradation of liquid cooking fumes experiment was carried out. Generally, the biodegradation efficiency of mixed dominant strains is higher than that of a single dominant strain (Wakelin and Forster 1997), so the four dominant strains 2xj-01, 2xj-02, jm-01, jm-02 and a mixture of strains were used to degrade liquid cooking fumes. The result shows that the mixture of strains has a higher rate of removal of liquid cooking fumes than that of any single strain, which agrees with the reported experiment. Figure 5 shows the change in removal rate of liquid cooking fumes using the mixture of strains under the optimum growth conditions (temperature at 37°C, the range of pH value at 6.0–7.0, 1.0 mg/L of inorganic salt, 5.0 mg/L of $NH_4H_2PO_3$ and 5.4 mg/L of dissolved oxygen) during the 32 h biodegrading reaction. In order to obtain reliable data, five series were carried out and every series was repeated twice. The initial concentration of liquid cooking fumes was 56.9, 74.6, 94.1, 105.6 and 135.6 mg/L, respectively. In all five series, all the initial concentration of microbe was 400 mg/L. It shows that, in each case, the removal of liquid cooking fumes increases with the reaction time.

Table 4 Results of orthogonal experiment

No.	A	B	C	D	E	Specific degradation rate (mg(oil)/mg(mass))
1	1	1	1	1	1	0.0236
2	1	2	2	2	2	0.0345
3	1	3	3	3	3	0.0255
4	1	4	4	4	4	0.0309
5	2	1	2	3	4	0.0240
6	2	2	1	4	3	0.0299
7	2	3	4	1	2	0.0313
8	2	4	3	2	1	0.0208
9	3	1	3	4	2	0.0287
10	3	2	4	3	1	0.0318
11	3	3	1	2	4	0.0341
12	3	4	2	1	3	0.0277
13	4	1	4	2	3	0.0329
14	4	2	3	1	4	0.0351
15	4	3	2	4	1	0.0138
16	4	4	1	3	2	0.023
k_{i1}	0.0288	0.0277	0.0283	0.03	0.0209	
k_{i2}	0.0278	0.033	0.026	0.0333	0.0296	
k_{i3}	0.0303	0.0224	0.027	0.0303	0.0281	
k_{i4}	0.024	0.0253	0.0314	0.0239	0.0317	
R_i	0.0063	0.0106	0.0054	0.0094	0.0108	

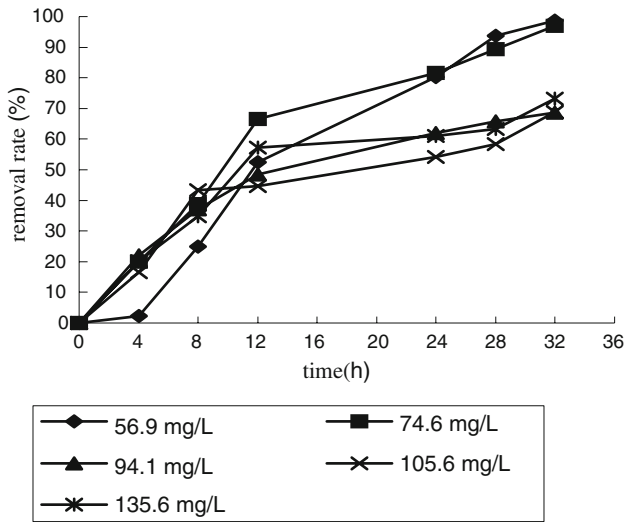

Fig. 5 Changes of remove rate in oil concentration

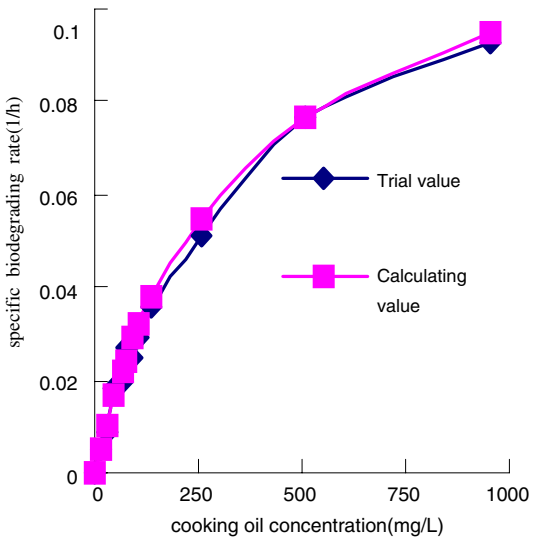

Fig. 6 Changes of specific biodegrading rate (SBDR) with cooking oil concentration

After 32 h reaction, the highest removal rate reached 98.6%, which indicates that the acclimatized microbes have a strong capability to degrade liquid cooking fumes. Figure 6 shows that the degrading reaction belongs to first-order biochemical reaction when the concentration of cooking fumes is very small, and the specific biodegrading rate is proportional to the concentration of cooking fumes. However degrading reaction belongs to zero order biochemical reaction when the concentration of cooking fumes is high, and the specific biodegrading rate has nothing to do with the concentration of cooking fumes. The reaction belongs to the transition region between first order and zero order reaction when the aqueous cooking fumes equal to the value of parameter K_S ($K_S = 352$ mg/L), and the specific biodegrading rate is equal to one half of the peak specific biodegrading rate (the value of $K = 0.13$ mg/L/h).

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