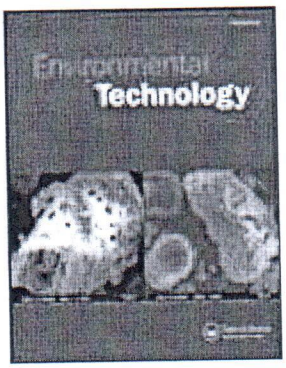

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Utilization of brewery wastewater for culturing yeast cells for use in river water remediation

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Utilization of brewery wastewater for culturing yeast cells for use in river water remediation

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Successful *in situ* bio-augmentation of contaminated river water involves reducing the cost of the bio-agent. In this study, brewery wastewater was used to culture yeast cells for degrading the COD_{Cr} from a contaminated river. The results showed that 15 g/L of yeast cells could be achieved after being cultured in the autoclaved brewery wastewater with 5 mL/L of saccharified starch and 9 g/L of corn steep liquor. The COD_{Cr} removal efficiency was increased from 22% to 33% when the cells were cultured using the mentioned method. Based on the market price of materials used in this method, the cost of the medium for remediating 1 m^3 of river water was 0.0076 US dollars. If the additional cost of field implementation is included, the total cost is less than 0.016 US dollars for treating 1 m^3 of river water. The final cost was dependent on the size of remediation: the larger the scale, the lower the cost. By this method, the nutrient in the brewery wastewater was reused, the cost of brewery wastewater treatment was saved and the cost of the remediation using bio-augmentation was reduced. Hence, it is suggested that using brewery wastewater to culture a bio-agent for bio-augmentation is a cost-effective method.

Keywords: reuse; brewery wastewater; bio-augmentation; river water; yeast

Introduction

Bio-augmentation is the application of indigenous or modified organisms to polluted sites or bioreactors, in order to accelerate the removal of undesired compounds. Yeast cells have always been used in bioaugmentation [1–4] because they are easy to grow, non-hazardous, robust, inexpensive, readily available and a significant component of the microbiota of most natural ecosystems [5–8]. The majority of these organisms have no known negative human health effects. Previous research studies have reported that *Candida* yeasts strains can be used to degrade polluted water [9–11]. To bioremediate the Waihuan River in Tianjin, China, different kinds of strains were isolated from the sediment of the river [12]. The yeast *Candida* CCTD-601 showed the best abilities for degrading chemical oxygen demand (COD_{Cr}), removing more than 30% of COD_{Cr} from the river water [13]; thus, it was selected for further application. Successful *in situ* bioremediation of contaminated surface water requires engineering a set of subsurface processes, in which the cost of the bio-agents is rarely considered; however, the cost cannot be neglected when the bio-augmentation is to be used for remediation of a broad area where a large amount of the agents are needed. Thus a less expensive method, to reduce bio-agent costs, needs to be considered.

The brewery industry has developed greatly alongside the improvement in people's living standards. In China, the total output of beer exceeded 11 million gallons in 2010, which meant that 11,220 million gallons of brewery wastewater was produced. The brewery wastewater is rich in nutrients, including protein, fat, fibre and carbohydrate, which can lead to big eutrophication problems when the wastewater is discharged into the environment. The treatment of brewery wastewater has received more and more attention [14–15]. The cost of the treatment is of concern, and economic treatment methods have been discussed [16,17]. Previous studies have found industrial waste products including whey, corn silage derivative, yeast, and spent sulphite liquor to be suitable carbon sources [18], which indicated that industrial wastewater containing rich nutrients could be used as the carbon source for culturing a bio-agent. According to that supposition, the cost of treating the brewery wastewater could be saved and meanwhile the nutrients in the wastewater could be reused. In a previous study, it was reported that brewery wastewater was used as a carbon source for production of flocculating agents, and the cost of the cultivation was reduced greatly [19].

The tested Waihuan River, which is close to the expressway around downtown Tianjin, China, and intersects with the Ziya and Haihe rivers amongst others, was designed to

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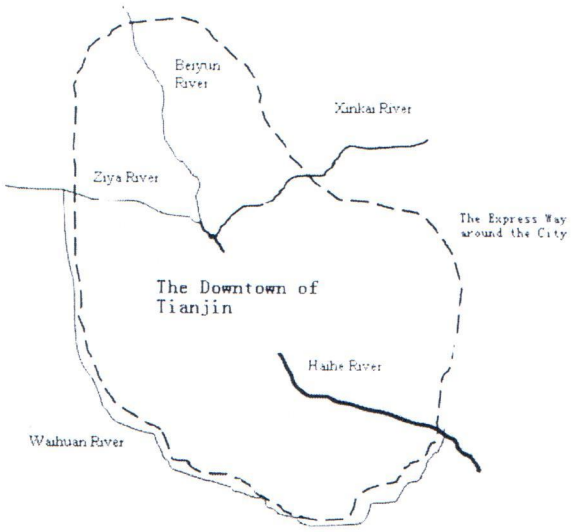


Figure 1. Map of the Waihuan River, which originates at the Ziya River and ends in the Haihe River. It was named 'Mother River' by citizens of Tianjin and plays an extremely important role in discharging floods, supplying water, transport, tourism and environmental protection.

drain rain water from the expressway and nearby farm land. The location of the river is shown in Figure 1. Unfortunately, some domestic sewage and industry wastewater was poured into the river, resulting in the deterioration of the water quality. The water quality of the river is as follows: NH_4-N 10–15 mg/L; TN 15–20 mg/L; COD_{Cr} 120–200 mg/L.

In this study, brewery wastewater was used to culture yeast cells. The cell growth and the COD degradation abilities, after being cultured in the brewery wastewater, were examined to test the feasibility of using the brewery wastewater and to obtain the optimal culturing method. It was found that the production cost of the bio-agent could be reduced and the cost of treating the brewery wastewater could be saved.

Materials and methods

Microorganism and medium

Candida CCTD-601 was previously isolated from sediments in the Waihuan River [12] and was noted to have a high COD_{Cr} reduction potential [13]. To ensure that the experiment condition was controllable, a synthetic brewery wastewater medium was used, which was designed as follows: beer: 15 mL/L; NH_4Cl: 120 mg/L; $MgSO_4 \cdot 7H_2O$: 100 mg/L; $FeCl_3 \cdot 6H_2O$: 0.5 mg/L; K_2HPO_4: 80 mg/L; KH_2PO_4: 50 mg/L; $CaCl_2$: 7.5 mg/L. The synthetic brewery wastewater was replaced with brewery wastewater once the optimal culturing method had been achieved. The brewery wastewater came from a brewery in Tianjin and consisted of 'out of date' and waste beer that was returned to the brewery and mixed with brewery washings in a balancing tank before biological treatment. Its characteristics were as follows: COD_{Cr}: 1000–2500 mg/L; biochemical oxygen demand (BOD_5): 700–1500 mg/L; suspended solids (SS): 200–500 mg/L; pH: 5–9.

Effects of the brewery wastewater on cell growth and COD_{Cr} degradation abilities

The first step was to test the feasibility of the brewery wastewater for culturing the yeast cells. The brewery wastewater was compared with the original medium. Additional carbon and nitrogen sources were added to the brewery wastewater to test the necessity of additional nutrients. The original medium included: sucrose 50 g/L, K_2HPO_3 5.0 g/L, $MgSO_4$ 2.0 g/L, $(NH_4)_2SO_4$ 3.0 g/L. The synthetic brewery wastewater was as mentioned above. For the sake of simplicity, the brewery wastewater supplemented with 10 g/L glucose and 5 g/L yeast extract was named 'M1', the original medium used to culture the yeast cells was named 'M2', and the pure brewery wastewater was named 'M3'. The seed cells were incubated in M1, M2 and M3 respectively. The cultivations were conducted in flasks with a shaking speed of 100 rev/min at 30 °C for 92 h. The exponential growth cells were harvested by centrifuging at 6000 × *g* for 5 min and washing three times with the autoclaved mineral medium. Finally the cells were diluted to 0.5 of OD_{620}. Two millilitres of the diluted cells were injected into 1 L of river water (COD_{Cr} = 146.51 mg/L), and were continuously cultured for nine days (according to the preliminary experiment). The supernatant was taken for measuring the removal of COD_{Cr}. Yeast-free samples were replicated as control. The experiment was repeated three times to ensure that the results were replicable.

Effect of the carbon source on cell growth and COD_{Cr} degradation ability

According to the result of the preliminary test, saccharified starch liquid was chosen as the additional carbon source. The starch was saccharified with liquefying amylase and maltogenic amylase by three steps: converting to a paste at 70 °C for 30 min, liquefying at 50 °C for 5 min and saccharifying at 40 °C for 24 h. According to the iodometric method, 2 g of glucose could be obtained from 1 mL of saccharified starch liquid; thus 5 mL/L of saccharified starch liquid was used, corresponding to 10 g/L of chemical glucose. Varying volumes of saccharified starch liquid (0.5, 1.0, 2.0 and 3.0 mL) were added to 100 mL of brewery wastewater with a pipette. The final concentrations of the saccharified starch liquid were 5, 10, 20 and 30 mL/L, respectively. Seed cells were inoculated in the brewery wastewater containing different concentration of saccharified starch liquid. The cultivations were conducted in flasks with a shaking speed of 100 rev/min at 30 °C for 90 h. The exponential growth cells were harvested to test their COD_{Cr} degradation abilities. The methods of harvesting, washing, resuspending,

dosing and sampling were same as the above-mentioned method. The COD_{Cr} of the river water in this experiment was 155.51 mg/L. The experiment was performed at least three times to ensure that the results were replicable.

Effect of the nitrogen source on the degradation ability and cell density

In this experiment, the corn steep liquor was compared with 5 g/L of yeast extract to test the feasibility and to obtain the optimal dosage. Steep liquor concentrations of 10, 20, 30 and 40 g/L were prepared by adding 1, 2, 3 and 4 g, respectively, of corn steep liquor to 100 mL of brewery wastewater before autoclaving. The corn steep liquor was obtained from Jiangsu (Xinyu, China). The cultivations were conducted in flasks with a shaking speed of 100 rev/min at 30 °C for 50 h. Later exponential growth cells were harvested to test their COD_{Cr} degradation abilities. The methods of harvesting, washing, resuspending, dosing and sampling were same as those mentioned above for the first step. The COD_{Cr} of the river water in this experiment was 175.69 mg/L. The experiment was performed at least three times.

Comparing the actual and simulated brewery wastewaters

Same amount of yeast seeds were inoculated in the brewery wastewater and synthetic brewery wastewater respectively. Both of the brewery wastewaters were supplemented with 5 mL/L of saccharified starch and 9 g/L of corn steep liquor. The cultivations were conducted in flasks with a shaking speed of 100 rev/min at 30 °C for 68 h. Later exponential growth cells were harvested by centrifuging at 6000 × g for 5 min and washing three times with the autoclaved mineral medium; finally the cells were diluted to 0.5 of OD_{620}. Two millilitres of the diluted cells were injected into 1 L of river water (COD_{Cr} = 157.89 mg/L) and were continuously cultured for nine days. The supernatant was taken for measuring the removal of COD_{Cr}. Yeast free samples were replicated as control. The experiment was tested in triplicate.

Analysis methods

Biomass concentrations were determined by measuring OD_{620} using the 752N ultraviolet spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, China). All of the ODs were read against the cell-free medium blank. Standard curves were constructed for yeast CCTD-601, to correlate OD_{620} values to dry weight. The standard curves were used to convert the OD_{620} measurements of unknown samples to dry biomass concentration.

The COD_{Cr} was analysed according to the potassium dichromate oxidation titration (GB 11914-89) [20].

Statistical analysis

The unpaired two-tailed student's t-test was used to compare sample values to the adjacent groups' values. Results with a T value of more than 0.05 were reported as significantly different.

Result and discussion

Effects of the brewery wastewater on COD_{Cr} degradation abilities of the cells

Although an equivalent number of yeast cells were cultured in the different media, the cells cultured in M1 could remove more COD_{Cr} than those in M2 and M3, and the cells cultured in M3 removed more COD_{Cr} than those cultured in M2 (T value = 0.0629: >0.05) as shown in Figure 2b. This demonstrated that the degradation activities were improved after culture in brewery wastewater. As reported by Zhang *et al.* [20], brewery wastewater was used as a carbon source to culture a multiple-microorganism consortia for producing a biofluocculant, which showed good flocculating performance in treating indigotin printing and dying wastewater. It was shown that the degradation abilities could be improved by being cultured in the brewery wastewater. We knew that the brewery wastewater contained a complicated nutrient

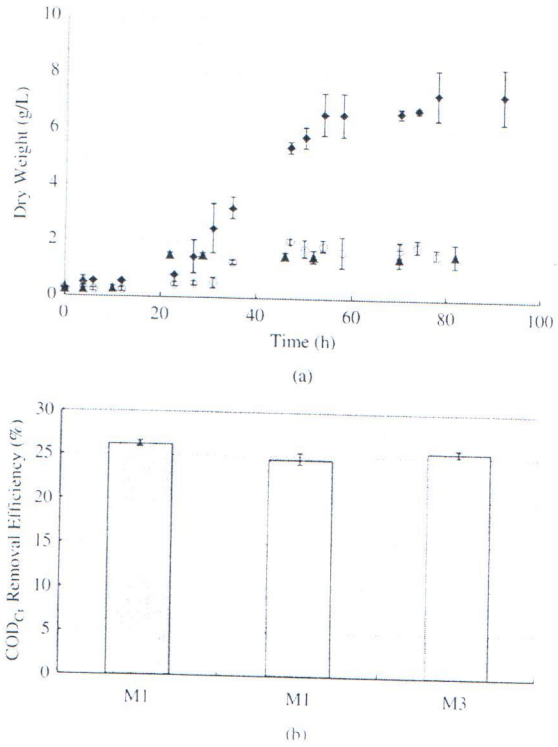


Figure 2. (a) Growth of yeast cells cultured in M1 (♦), M2 (○) and M3 (▲). (b) COD_{Cr} removal efficiency of the cells pre-cultured by M1, M2 and M3 media (COD_{Cr} of the river water in this study was 146.51 mg/L). Typical results from more than three experiments are shown. Error bars: ± S.D. of the replicates.

mix, some of which may stimulate the activity of yeast cells. Further studies on the mechanism of stimulation should be conducted in future research. As seen above, brewery wastewater can be used to culture the yeast, and degradation activities can be improved. The cell density can be increased (Figure 2a) and the degradation abilities can be improved when additional carbon source and nitrogen source are added; thus this additional carbon source and nitrogen source was necessary.

Effect of the carbon source on the COD_{Cr} degradation ability

Starch is a polysaccharide composed of α-glucose units that are linked by α-1,4- and α-1,6-glycosidic bonds, forming two high-molecular-mass molecules including amylose (15–25%) and amylopectin (75–85%). It was not easily used by the yeast cells unless it was saccharified. After the saccharification process, not only was the glucose produced but also the branched oligosaccharides panose, isopanose and isomaltose were usually formed [21] and peptone was also produced by the protease. The dry weight of the yeast cells were improved from 6.5 g/L to 8.26 g/L and the COD_{Cr} remove rate was increased from 26.66% to 31.5% when the cells were cultured in the medium where saccharified starch was used as the carbon source, compared with the cells cultured in the medium where glucose was used as the carbon source (Figure 3). This indicated that the saccharified starch could be used as the carbon source to culture the yeast cells without decreasing their degradation abilities. The by-products produced in the saccharification process may be good for the growth of the yeast cells and their degradation abilities. Ejiofor *et al.* [22] established that cassava starch hydrolysate was a suitable low-cost replacement for glucose in the production of baking-quality yeast [22]. However, when the addition of saccharified starch was doubled from 5 ml/L to 10 ml/L, the cell concentration was increased by only 1 mg/L. There was no statistically significant difference ($T < 0.05$) in the degradation abilities, which implied that the effect of increasing the amount saccharified starch on the cell growth was negligible and the degradation ability was independent of the dosage.

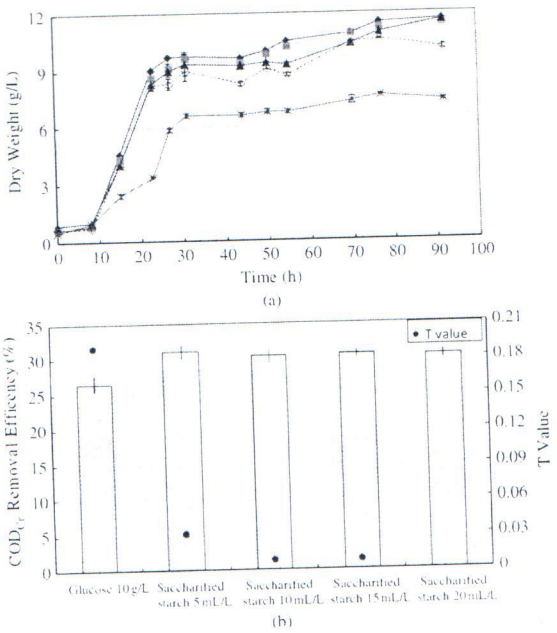


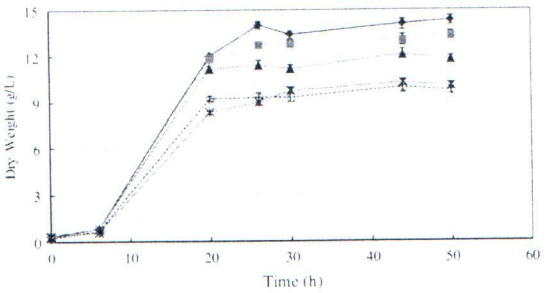
Figure 3. (a) Growth of yeast cells in the medium with different concentrations of saccharified starch added to the brewery wastewater: —+— 10 g/L of glucose; ····◇···· 5 mL/L of saccharified starch; —▲— 10 mL/Lof saccharified starch; – ■ – 15 mL/L of saccharified starch; —●— 20 mL/L of saccharified starch. (b) COD_{Cr} removal efficiencies by the same amount of cells that cultured with different carbon sources (COD_{Cr} of the river water in this experiment was 155.51 mg/L); • is the T value between adjacent groups. Typical results from more than three experiments are shown. Error bars: ± S.D. of the replicates.

Effect of the nitrogen source on the COD_{Cr} degradation ability

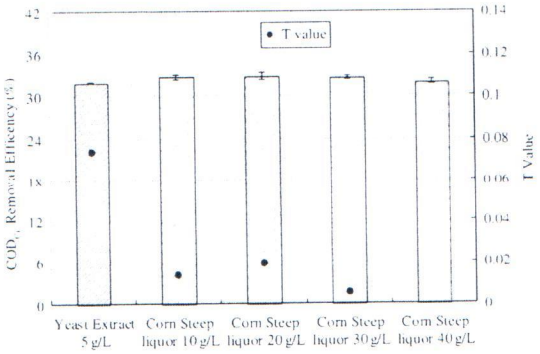
Corn steep liquor is a good and inexpensive alternative to other materials such as yeast extract and peptone, and has been utilized by many researchers [23–26]. In this study, corn steep liquor was used to culture the yeast cells, and the concentration of the corn steep liquor was studied to improve the cell density while not destroying the degradation abilities of the cells. In Figure 4, 9.19 mg/L of yeast cells were obtained after being cultured in the 10 g/L corn steep liquor medium, whereas 8.27 mg/L of yeast cells were obtained when cultured in the medium added with 5 g/L of yeast extract. The COD_{Cr} removal rate was 32.69% for the cells cultured in the medium with 10 g/L of corn steep liquor, whereas the COD_{Cr} remove rate was 31.98% for the cells cultured in the medium supplemented with 5 g/L of yeast extract. Although there was no standard method to evaluate the differences between the 5 g/L of yeast exact and the 10 g/L of corn steep liquor, the T value of 0.074 (>0.05) between the two groups for COD_{Cr} removal rate showed that an improvement in degradation abilities existed, implying that the degradation ability was stimulated by the addition of corn steep, and the corn steep liquor could be a replaceable nitrogen source. However, the degradation ability decreased in the samples where more than 10 g/L of corn steep liquor was added. The mechanism was complicated, and the factors influencing the degradation abilities of the cells were interactive. Thus an optimizing mathematical method was needed to determine the optimal concentration.

Optimizing the culture condition with a mathematical method

In field bio-augmentation, not only is a high concentration of cells needed, but also the COD_{Cr} degradation abilities need to be protected or improved. Thus an optimizing



(a)



(b)

Figure 4. Growth of yeast cells cultured in 5 g/L of yeast extract yeast (—+—) and different concentrations of corn steep liquor: ----○---- 10 g/L; —▲— 20 g/L; —■— 30 g/L; —◆— 40 g/L. as added and the COD_{Cr} removal efficiencies by the same amount of cells that cultured with different nitrogen source. (b) COD_{Cr} removal efficiency by the cells pre-cultured with different nitrogen source (COD_{Cr} of the river water was 175.69 mg/L); • is the T value between adjacent groups. Typical results from more than three experiments are shown. Error bars: ± S.D. of the replicates.

Table 1. Orthogonal designs.

Saccharified starch (mL/L)	Corn steep liquor (g/L)	Medium volume (mL/250 mL)	Inoculation amount (%)	pH
1	6	40	5	4.5
3	9	60	10	5.0
5	12	100	15	5.5
7	15	120	20	6.0

mathematical method with multiple objectives [27] was developed. The orthogonal experimental method was used to design the optimizing experiment, where the concentrations of saccharified starch and corn steep liquor, volume of medium, inoculation amount and pH value were varied, as shown in Table 1.

The two objectives, to produce a high concentration of cells and to protect/improve COD_{Cr} degradation ability, could not be calculated together for they had different dimensions, so the mapping function in fuzzy mathematics was used to analyse the data. According to the method, every index was plotted in the [0, 1] value space, then the index was determined according to their importance in the overall merit. The values of the concentration and degradation ability were defined respectively as $b_1 = 40$ and $b_2 = 60$. The computing of Y is shown in Equation (1).

$$Y = \{b_1\}\{Y_{j1}\} + \{b_2\}\{Y_{j2}\}\sum_{i=1}^{2}\{\{b_i\}_j\{Y_{ji}\}\} \qquad (1)$$

where Y is the comprehensive weighted value, j is the experiment group number and i refers to the objective parameters (the i-value of dry weight and remove efficiency are named 1 and 2, respectively). According to the previous result, the final dry weight was about 0–20 g/L and the removal efficiency was about 0–50%. The mapping function of types is shown in Equation (2).

$$Y_{ji} = \begin{cases} 1 & x_{ji} < c \\ 1 - 2\left[\dfrac{x_{ji} - c}{a - c}\right]^2 & c \le x_{ji} < b \\ 2\left[\dfrac{x_{ji} - c}{a - c}\right]^2 & b \le x_{ji} < a \\ 0 & x_{ji} \le a \end{cases} \qquad (2)$$

The values of parameters a, b and c are shown in Table 2. The Y value was the final result to determine the optimal method. In addition, the results in Figures 2–4 have shown that removal efficiency was not improved even when more saccharified starch or corn steep liquor was added. Using less material would lower the costs. Thus, the optimal conditions were as follows: stimulated brewery wastewater: 1 L; saccharified starch: 5 mL; corn steep liquor: 9 g; medium volume: 100 mL/250 mL; inoculation amount: 15%, pH value 4.5.

After cultivation, the residual nutrient levels were as follows: COD_{Cr}: 8000 mg/L; BOD_5: 3000 mg/L; NH_3-N: 250 mg/L. By using the above cultivation method, because brewery wastewater can be used to culture the yeast cells, the treatment cost of the brewery wastewater can be saved. However, the wastewater after fermentation needs to be further treated, which is same for other cultivation methods.

Comparison of the brewery wastewater and the synthetic brewery wastewater

There are always differences between a synthetic brewery wastewater and an actual brewery wastewater. The optimal method determined with the synthetic brewery wastewater

Table 2. Evaluated values of the parameters in Equation (2).

	Index		
Parameters	*a*	*b*	*c*
Dry cell weight (g/L)	20	10	0
Remove efficiency (%)	50	25	100

needed to be verified by comparing with an actual brewery waste water. The growth curve of the cells cultured in the brewery wastewater was similar to that of the cells cultured in the synthetic one (Figure 5a), which meant that the characteristics of the synthetic wastewater were close to those of the actual brewery wastewater for culturing the yeast cells. The yeast cells cultured in real brewery wastewater could remove 35% of the COD_{Cr}, whereas the same amount of cells cultured in the synthetic wastewater could remove 32% of the COD_{Cr} (Figure 5b), which indicated that the actual brewery wastewater can stimulate the degradation abilities better than the synthetic wastewater. Thus, the actual brewery wastewater could be used to culture the yeast cells used for degrading the COD_{Cr} of river water. A comparison with the results reported by Liu [28], where a COD_{Cr} removal efficiency of 30% was achieved when a commercial bio-agent was used, indicates that not only the culturing cost could be saved but also an improved COD_{Cr} removal efficiency could be achieved using brewery wastewater.

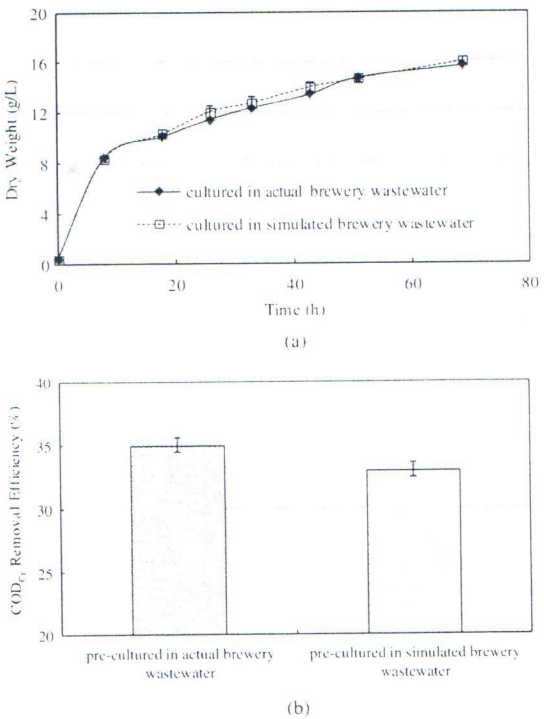


Figure 5. Comparison of actual and simulated brewery wastewater in terms of (a) cell growth and (b) COD_{CR} degradation abilities of the cultured yeast. Typical results from more than three experiments are shown. Error bars: ± S.D. of the replicates.

Economic analysis

The cost of culturing the yeast cells was calculated according to the market price of materials used in this study, including corn steep liquor, starch, liquefying amylase and maltogenic amylase. In addition, the cost of energy and labour for culturing was also considered according to the current costs in China. For a dosage of 2‰ (v/v) (the concentration of the cells was 15 g/L), the cost of the medium for remediating 1 m^3 of river water was 0.0076 US dollars (Table 3). In a field experiment, the cost should also include (1) the cost of transportation from the culturing place to remediation field, (2) the cost of labour, equipment, energy and so on. According to the current field experience in Waihuan River, the additional cost is less than 0.0083 US dollars for remediating 1 m^3 of river water. Academically, the total cost will depend on the size of the remediation, where a larger size would mean lower cost. This method has been used in the field. The cultured yeast cells were washed and dosed back into the river. It was shown that 20–40% of the COD_{Cr} could be removed and the total cost was less than 0.016 US dollars for remediating 1 m^3 of river water.

Conclusions

Brewery wastewater was used to culture yeast cells to remediate polluted rivers. The feasibility of using the brewery wastewater and its effects on the yeast's cell growth and COD degradation activities were discussed. It was demonstrated that brewery wastewater could be used to culture the yeast, and the COD degradation potential of the yeast could be improved after being cultured in the brewery wastewater medium.

The growth and the degradation ability could be improved by adding saccharified starch and corn steep liquor. These are inexpensive alternatives to much more expensive materials.

By using this method, the cost of the culturing medium was 0.0076 US dollars for remediating 1 m^3 of river water. According to the field experience, the total cost always depended on the size of the remediation, with a larger size corresponding to lower costs.

Because of the reuse of brewery wastewater as the carbon source, the cost of treating the brewery wastewater was

Table 3. Cost of medium prepared with brewery wastewater.

	Saccharified starch	Corn steep liquor	Brewery wastewater	Total
Amount of used materials	5 mL	9 g	1 L	
Unit price, US dollar/kg	0.676	367.65	0	
Agent cost, US dollar/L	4.97×10^{-4}	3.31×10^{-3}	0	0.0038
Cost of medium for treating 1 m^3 of river water, US dollar				0.0076

saved and the cost of remediating the river was reduced. It was thought to be an economic method.

In this research the used yeast was isolated from the tested river, thus no exterior microorganism was involved and it was thought to be safe for the ecology of the river; however, further research should be conducted to ensure the safety of the added bio-agents.

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