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Research Article

Formaldehyde-Degrading Bacteria R1 Is Effective in Removing HCHO from the Air in an Indoor Environment

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Formaldehyde (HCHO) is a widespread air pollutant in the indoor environment. Previous studies have shown that some bacteria have potential application to remove indoor HCHO. The purpose of this study is to evaluate the effectiveness of *Methylobacterium* sp. strain R1 (S-R1) in removing formaldehyde (HCHO) from indoor air using biofilters. Three experiments confirmed S-R1's ability to degrade HCHO in the air, with ^{13}C-NMR analysis revealing its involvement in the metabolic process. Optimal biofilter parameters, including 35 sponge layers, 30% humidity, and 9.50 m^3/min air flow, resulted in a removal efficiency of up to 90% and an elimination capability of 24111-27000 $\mu g/(m^3 * h)$ during a 60-minute test period. Long-term (31-day) operation of the biofilter with the optimal parameters effectively reduced HCHO levels from 1.60 mg/m^3 to 0.02-0.03 mg/m^3, below China's national standard, and maintained this level. Fluorescence microscope observation and downstream gas detection revealed stable S-R1 cell numbers and no bacterial leakage, respectively. Two conclusions can be drawn: (1) S-R1 is effective in removing HCHO in polluted air and (2) with optimum parameters, the S-R1 biofilter is engineering effective in purifying the indoor air environment.

1. Introduction

Formaldehyde (HCHO) is a widespread air pollutant in the indoor environment. HCHO pollution is found in indoor air of newly renovated houses, chemical and pharmaceutical workshops, organic reagent repositories, and chemical laboratory rooms [1]. People living and working in such environments are often exposed to HCHO pollution [2]. Exposure to HCHO can cause a variety of abnormalities and diseases of human body [2] including cancer, and HCHO is considered as class one carcinogen. HCHO is as well an endogenous metabolite present in all organisms [3].

Physical and chemical methods are the most popular technologies currently used for the removal of indoor HCHO pollution. However, the disadvantages of these methods are obvious: high cost, complex devices, and lack of sustainability. For example, one physical method to remove HCHO is to absorb it through porous materials, but the capability is limited after saturation. Chemically, the photocatalytic oxidation technology is widely used, but the efficiency of removing HCHO is not ideal because of the photocatalyst activity is usually not satisfiable.

A spectrum of organisms have formed their own mechanisms of HCHO detoxification during evolution to resist the lethal and mutagenic effects of HCHO, including plants [4–8] and microorganisms (bacteria and fungi) [9–14]. Intensive researches focused on HCHO-degrading bacteria and found that the types of this kind of bacteria included

Pseudomonas putida, *Pseudomonas aeruginosa*, *Methylobacterium* twisted, *Pseudomonas pseudoalcaligenes*, *Pseudomonas testosterone*, and methylotrophic bacteria [15–20]. And among these kinds of bacteria, methylotrophs appeared to have more complex and effective HCHO metabolism systems [13].

In recent years, more and more researches have focused on biological method to remove HCHO pollution based on these investigations. Researchers believe that using microorganisms or plants is a simple, economic, and ecofriendly solution. The engineering-designed biofilters have the advantages of low cost of running, low pressure of the instrument, large volume, and no secondary pollution [21].

Previous studies have already explored a variety of bacteria with different constructions of biofilter that can remove and degrade HCHO from the polluted air or wastewater [22–29]. However, many of them lacked engineering evaluation of the efficiency of removement with different constructions of working parameters, such as the density or number of bacteria used, the intensity of air flow of the biofilter, or the humidity of the bacteria living environment.

In the current study, we used a strain of bacteria named *Methylobacterium* sp. strain R1, which was derived from our previous research, to construct a prototype of biofilter for degrading HCHO-polluted air in a limited space as well as in a polluted room. The purposes of the current study are as follows: (1) to investigate whether this specific bacteria could degrade HCHO in air while we previously found that they were effective in removing HCHO in polluted water; (2) to select an optimum combination of working parameters (air flow rate, humidity, and volume of bacteria medium) to generate best effects for the prototype of biofilter; and (3) to investigate whether the optimal constructed biofilter engineering effective in purifying the air in an indoor environment of a HCHO-polluted room.

2. Methods and Materials

2.1. Preparation of Bacteria and Core Part of Biofilter

2.1.1. Strain Source of Bacteria and Culture Medium. The formaldehyde-degrading bacteria used in this study was a *Methylobacterium* sp. strain R1 (S-R1, GenBank: MK618640) which was derived from the wastewater sludge contaminated by HCHO [30]. In this study, two bacterial species were isolated by growing on a formaldehyde-containing agar medium. And biochemical tests showed that one of the strains was able to use HCHO as the sole carbon source and could be identified as *Methylobacterium* with a capability of 20 mM for degrading HCHO [30].

The strain of bacteria was cultivated in the inorganic medium which is composed of ammonium chloride 141.5 mg/L, magnesium sulfate 19.5 mg/L, calcium chloride 22.8 mg/L, ferrous sulfate 6 mg/L, potassium dihydrogen phosphate 35 mg/L, dipotassium hydrogen phosphate 45 mg/L, and trace element solution (1 m L/L). A small amount of methanol (0.5%, w/w) was added to the inorganic medium; then, the S-R1 was placed into the medium which was exposed in the air at 25°C for 5-6 days. After the OD_{600} of the bacteria reached ~1, an equal volume of medium was added and mixed thoroughly to obtain the diluted S-R1 solution.

2.1.2. The Core Part of Biofilter: Sponge with S-R1. Sodium alginate (1.0%, w/w) and carboxymethylcellulose (CMC 2%, w/w) were mixed with distilled water and were stirred in a beaker at 55°C for 12 hours to be fully dissolved. Then, this solution was cooled to room temperature (22°C) and was mixed with an equal volume of diluted S-R1 solution obtained from above 1.1 part. The mixed solution was prepared as the embedding solution.

The synthetic polyurethane sponge (lower intensity type with average ~0.5 mm of pores and 0.5 cm thickness) was cut into pieces ($28 \times 18\ cm$) and placed in a plastic basin. Then, the sponge was emerged into the embedding solution, and let the solution fully moisturize the sponge pores. The sponge was then transferred into 2% $CaCl_2$ solution for 10-20 minutes to perform a reaction in which the Na^+ in the sodium alginate was replaced with Ca^{2+}. Therefore, S-R1 cells were permanently embedded into the sponge net. The sponge was placed in tap water for 10-30 minutes to solidify and then was air-dried to humidity to contain just 40-50% of water. Finally, the sponge was placed into a plastic bag and was stored in a cool room with the temperature of 7-8°C for using in an instrument in the next stage of experiment.

To verify whether S-R1 was actually fixed on the sponge, a scanning microscopy was used to observe the microstructure of the sponge after the experiment. Compared with the sponge emerged with saline water (microstructure is shown in Figure 1 upper right panel), the S-R1 were successfully embedded on the net grid, shown as the orange disks which were the bodies of bacteria in the lower right panel of Figure 1. This means the sponge with S-R1 did serve as the core part of the biofilter in current study.

2.2. To Investigate Effectiveness in Air Treatment: Experiment 1

2.2.1. Instrument. A simplified instrument, consisting 2 sealed boxes, served as a passive biofilter to investigate if S-R1 is effective in decreasing HCHO in the air (Figure 1). Also, in order to confirm that S-R1 was actually involved in the process of decomposing HCHO, an ^{13}C labeling H-^{13}C-HO solution was used in this experiment. There was a cup with cotton dipped with $H^{13}CHO$ solution as the evaporator of $H^{13}CHO$ in plastic box 1 ($20\ cm \times 30\ cm \times 25\ cm$, $0.015\ m^3$). In box 2 ($35\ cm \times 30\ cm \times 25\ cm$, $0.026\ m^3$), a piece of moisturized sponge was placed on the bottom of the box to serve as the main part of the biofilter. In the experiment condition, the sponge was emerged with S-R1 with base liquid, while in control condition, the sponge was moisturized with saline water. A plastic pipe connected the two boxes with a valve on it, and on the end of the pipe in box 2, a fan was attached to blow the air from box 1 into box 2. There are 2 formaldehyde density tester (PPM htV, PPM Technologies Co., Ltd., England) attached on each of the box with the sensor tip in the boxes. Before the experiment starts, the valve on the pipe was kept off; the air in box 2 contained no $H^{13}CHO$. When the

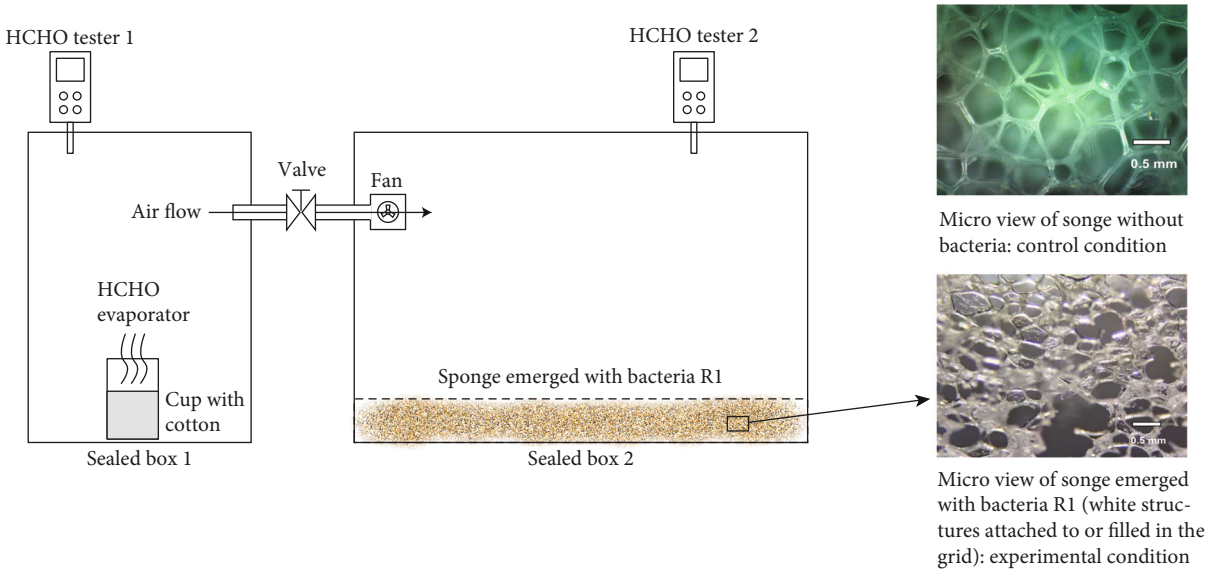

Figure 1: The instrument for experiment 1. Two sealed boxes were connected with a pipeline, and a fan was attached on the end of the pipeline in box 1 which can blow the air into box 2. A valve on the pipeline could control the air flow from box 1 to box 2. A cup with cotton containing HCHO was placed in box 1 to serve as HCHO evaporator to supply the HCHO which could be pumped into box 2. A layer of sponge emerged with bacteria R1 was placed on the bottom of box 2 to serve as the simplified biofilter of HCHO. Two HCHO testers were attached to the boxes, respectively, to measure the concentration of HCHO in the air of each of the boxes. For experimental condition, the sponge was emerged with bacteria R1 (the microview of the lower right panel shows the bacteria attached on the grid of the sponge), while for control condition, the sponge was emerged with pure water without bacteria (microview shown in the upper right panel).

experiment began, the valve was turned on until the read of $H^{13}CHO$ density in box 2 reached 59.88 mg/m^3 (2 mM), and then, the valve was off again, but the fan in box 2 was continuously on to fully mix the air in the box. The whole experiment lasted for 24 hours, and the read of the tester 2 attached to box 2 was recorded 2 and 4 hours after the experiment started and then every 4 hours since after. For each data point, the read of the tester was recorded 3 times in 3 minutes, and the mean value was calculated as the density of HCHO of that time point. During the whole experiment, the temperature in the room as well as in the boxes was maintained at 25°C.

2.2.2. $H^{13}CHO$ Labeling Evaluation of ^{13}C-NMR Analysis for Metabolites of S-R1. It is necessary to investigate whether S-R1 was actually involved in the process of decomposing HCHO in experiment 1. To confirm this, the ^{13}C labeling H-^{13}C-HO substance was used for in this experiment, and after the experiment was finished, NMR analysis was performed for the evaluation of metabolites of S-R1. After the experiment, the sponge was air-dried at 55°C in a container and then was cut off into small pieces. A portion of small pieces were placed into 25 mL volume of potassium phosphate buffer (KPB, 10 mM, pH 7.4) to extract the S-R1 cells from the sponge pieces. Then, the solution was treated by sonication to break the S-R1 cell bodies and was boiled for 3 minutes and centrifuged at 12,000 rpm for 10 minutes to remove the cellular debris. The separated supernatant was frozen, lyophilized, and resuspended in 0.5 mL of KPB containing 5% (v/v) ^2H_2O and was filled into a 5 mm NMR tube for NMR analysis. The sponge, which contained S-R1 but was not exposed to HCHO (or $H^{13}CHO$) and was kept in regular air, underwent the same treatment procedure as the experimental group. This sponge served as the control group, intended for detecting and comparing the background ^{13}C signals of the R1 cells.

The ^{13}C-NMR analysis was performed on a Bruker DRX 600 MHz instrument (Bruker Biosciences Corporation, Billerica, MA). A 50 mM formamide was added into the sample as the reference (Ref). ^{13}C-NMR data were collected according to previously described parameters [31]. The acquisition parameters included a 5 μs (90°) pulse with broadband proton decoupling, a spectral width of 37,594 Hz, an acquisition time of 0.5 s, and a decay time of 1.2 s. The sample temperature was maintained at 25°C, and 32,000 data points were acquired for each sample. Twelve hundred scans were acquired for each sample, and a line broadening of 4 Hz was used to process the data. In the ^{13}C-NMR spectra, the chemical shifts were referenced to the resonance of Ref at 166.65 ppm. The ^{13}C-NMR spectra were calibrated relative to the reference to compare the interesting peaks in different samples. The resonance peaks were assigned through comparisons with the authentic compound's chemical shifts and confirmed by spiking the extracts with the authentic reference standards. To compare the relative contents of the metabolites, the target peaks were integrated relative to the Ref peak (set as 1).

2.3. Evaluation of Performance for Biofilter of S-R1: Experiment 2

2.3.1. Instrument. A prototype of biofilter was made for this experiment (Figure 2). The major parts of the instrument

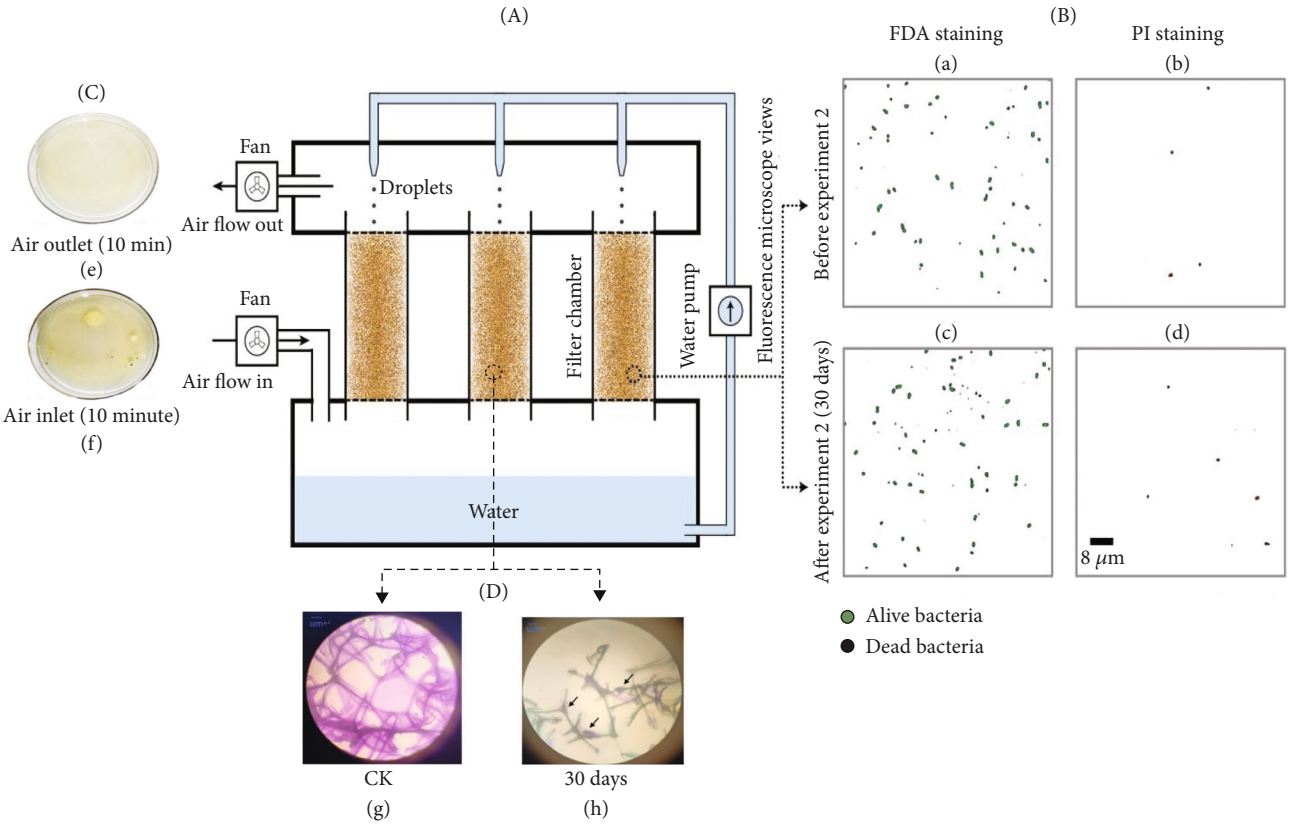

Figure 2: (a) Instrument of biofilter for experiment 2. The filter chambers were fixed between a base tank and an upper box. The base bank contained saline water which could be pumped into the upper box through pipelines to form water droplets to moisturize the filter chambers to keep the bacteria alive. The filter chamber was filled with sponge with bacteria R1, the same to the sponge in experiment 1. Air was blown into the based tank, flowed through the filter chambers, and then fanned out through upper box. The whole instrument was placed in a sealed room in which the density of HCHO was originally measured as 1.76 mg/m^3. (b) The fluorescence microscopic views of (A, C)FDA staining for alive bacteria and (B, D) PI staining for dead bacteria in the sample sponges before experiment 2 (A and B were separated from a same view) and after 30 days when experiment 2 was finished (C and D were separated from a same view). (A) 63 counts of alive bacteria R1 (in green dots) before experiment. (B) 4 counts of dead (in red dots). (C) 67 counts of alive after experiment. (D) 6 counts of dead. (c) Microbial growth status of inlet and outlet of biofilter. (d) The microscopic images of samples withdrawn from the biofilter. Observation of the sponge embedding R1 cells (h) after 30 days and the control (CK) sponge (without embedded R1 cells) (g) under a scanning electron microscope. Microbial growth status of inlet and outlet bacteria in filter bioreactor (e and f) after 10 minutes of treatment.

were 15 filter chambers, and each chamber was a cylinder (10 cm in height and 5 cm in diameter) filled with moisturized sponge with S-R1 in it. The filter chambers were fixed on the base tank ($40\,cm \times 30\,cm \times 20\,cm$) and connected to an upper box ($20\,cm \times 30\,cm \times 10\,cm$) with fans fixed on them with a 3 switches of air flow of 9.50, 7.12, and 4.75 m^3/min. The air in the room can only be blown into the base tank, flew through the filter chamber, and then went back into the room from the upper box. The base tank contained saline water which could be pumped onto the upper box through pipelines forming droplets dripping into the filter chambers which contained humidity sensors to switch on/off the water pump to keep the humidity of the sponge in the filter chambers in a constant range.

2.3.2. Evaluation of Removal Efficiency and Elimination Rate of HCHO. For this experiment, the instrument of biofilter (Figure 2) was placed in an empty room, with the size of 4 $m \times 5\,m \times 3\,m$ and about 60 m^3 empty space in it. The initial HCHO density was tested as 0.02 mg/m^3, so a cup of 37% HCHO solution was placed in the center of the room to obtain a 1.00 mg/m^3 of HCHO density in the air. Then, the HCHO solution was removed from the room, the instrument of biofilter was turned on, and the door was sealed during experiment. In current experiment, the HCHO tester was placed across to the biofilter and a fan was placed in a corner of the room to ensure that the air was fully mixed. The read of the HCHO tester was recorded every 5 min through Wi-Fi signal; thus, a total of 12 reads was recorded in each session which lasted for 60 min. A total of 9 sessions were finished in the current experiment. In the first 3 sessions, the number of layered sponge was filled with 35 pieces in the cylinder (Figure 2), the flow of air by fans was set at 9.50 m^3/min, and then, the humidity in the cylinder was controlled at 18%, 30%, and 52%, respectively. In the second 3 sessions, the sponge layers were kept at 35 pieces and the humidity fixed at 30%, while the air flows were set at 4.75, 7.12, and 9.50 m^3/min, respectively. In the last 3 sessions, the sponge layers were changed as 15, 25, and 35 pieces while the humidity was kept at 30% and the air flow at 9.50 m^3/min.

The HCHO removal efficiency (RE) was calculated to evaluate the performance of the biofilter [32]. The RE was defined as follows:

$$\mathrm{RE} = \frac{C_{\mathrm{in}} - C_{\mathrm{out}}}{C_{\mathrm{in}}} \times \%, \quad (1)$$

where C_{in} and C_{out} refer to the HCHO density ($\mathrm{mg/m^3}$) in the in and out air of the biofilter, respectively.

The HCHO elimination capability (EC, $\mathrm{g/(m^3 \cdot h)}$) of the biofilter was calculated as follows:

$$\mathrm{EC} = Q \times \frac{C_{\mathrm{in}} - C_{\mathrm{out}}}{V}, \quad (2)$$

where Q is the air flow rate ($\mathrm{m^3/min}$) and V is the volume of the sponge packing in the cylinder in the biofilter ($\mathrm{m^3}$).

2.4. To Investigate Effectiveness to Purify Air in a HCHO-Polluted Room: Experiment 3

2.4.1. Environment. The room for this experiment was empty with the size of about $4\,m \times 5\,m \times 3\,m$ with roughly $60\,\mathrm{m^3}$ empty space in it. This room previously served as a storage room for chemicals without any windows in it and had just painted and decorated not long before. The room had been kept closed since renovation, and the HCHO was measured up to $1.76\,\mathrm{mg/m^3}$ in natural state, much higher than the standard level for indoor air by the government where this experiment was performed ($0.08\,\mathrm{mg/m^3}$, GB/T 18883-2022). So this room could ideally serve as a typical example of a HCHO-polluted indoor environment. The room was carefully sealed, and during the whole period of experiment in 30 days, no access was allowed. The instrument was monitored by a camera and cellphone. The read of the tester was recorded every day at noon by an app connected to the tester through Wi-Fi signal.

2.4.2. Survival Rate Evaluation of S-R1 in the Sponge of the Biofilter. Fluorescein diacetate (FDA) is a dye that can be hydrolysed by esterase in living cells. FDA can be activated by blue light at wavelengths of 450-490 nm and produces bright green fluorescence, which reflects the survival of living cells [33]. Propidium iodide (PI) is a fluorescent dye that is used to stain nuclei and chromosomes. PI-stained dead cells produce red fluorescence when observed under a fluorescence microscope. Because PI cannot be taken by living cells, it is commonly used to detect the dead cells in a population [34, 35].

After the biofilter operated for 30 days when experiment 2 had finished, a piece of sponge strip was taken out from the filter and was cut into small pieces and soaked in sterile water. The S-R1 cells were extracted into the water to form solution. A drop of the solution was put onto a glass slide and added with a small amount of PI (1 mg/mL) and FDA (5 mg/mL) for staining at room temperature and kept in a dark room for 5 minutes. Then, the glass slide was put under a confocal laser scanning microscope (A1, Nikon, Japan) with Kr/Ar laser excitation with ~495 nm for activating FDA fluorescence and ~540 nm for PI, while the emission wavelengths were ~510 nm and ~625 nm, respectively. The number of living bacteria (in green) and dead (in red) was counted in the scope of the microscope to evaluate the robustness of the bacteria during a month for this experiment.

2.4.3. Microscopic Images of Samples and Microbial Detection at Inlet and Outlet. Biomass samples were graphed using optical microscope. The photographs of the packing material were taken before and after 30 days of operation in the bioreactor.

The use of bioreactor to purify indoor air, the most important problem is that the bioreactor will not discharge microorganisms into the room, and can ensure environmental safety. Detection of downstream microbial leakage is therefore necessary for potential indoor applications of biofilters. In this experiment, we collected inlet and outlet gas and treated LB agar plate with it. During this period, the plate cover is opened and placed in inlet and outlet position and then treated with gas for 10 minutes.

2.5. Data Analysis. For the grouped data in the above experiments, T-test was used to test the difference between groups with a licensed Matlab (R2018a). Significant level was set at $P < 0.05$.

3. Results

3.1. Experiment 1: S-R1's Effectiveness in Air Treatment. When the density of HCHO in box 2 for the initial state had reached to $59.88\,\mathrm{mg/m^3}$, the valve was closed and the experiment began. For the first measurement at the time of 2 h, the density of HCHO was $23\,\mathrm{mg/m^3}$, almost reaching 1/3 of the initial state (Figure 3(a)). The density constantly decreased to $12\,\mathrm{mg/m^3}$ at the time of 24 h for experiment condition. Compared to the control condition with sponge containing no S-R1, the first read of the tester was $56\,\mathrm{mg/m^3}$ and then decreased to $37\,\mathrm{mg/m^3}$ at 24 h, about 3 times of the experiment condition at the same time in end of the experiment. The T-test showed great significance ($P < 0.001$) between experiment condition and control condition for a total of 6 measurements from 2 h to 24 h for each condition (Figure 3(b)).

The ^{13}C-NMR data show that a variety of signals of compounds in S-R1 cells had changed during time in the process of the experiment (Figure 4 and Table 1). The baseline of intensity of different compounds is shown on the top line of "Before Exp" in Figure 4, and changes 2 h and 24 h after the experiment started were shown in the second and third lines, respectively. The results suggest that $\mathrm{H^{13}CHO}$ in the R1 cells might be oxidized into formate and assimilated into sugars and other organic acids and amino acids. This may suggest that the HCHO metabolism in the embedded R1 cells had played an important role in the HCHO removal.

3.2. Experiment 2: Evaluation of Performance. With 35 pieces of sponge in the cylinder and the air flow at $9.50\,\mathrm{m^3/min}$, the HCHO removal efficiency in the first 3 sessions was continuously higher in 30% of humidity across 60 minutes than that the humidity set at 18% and 52%

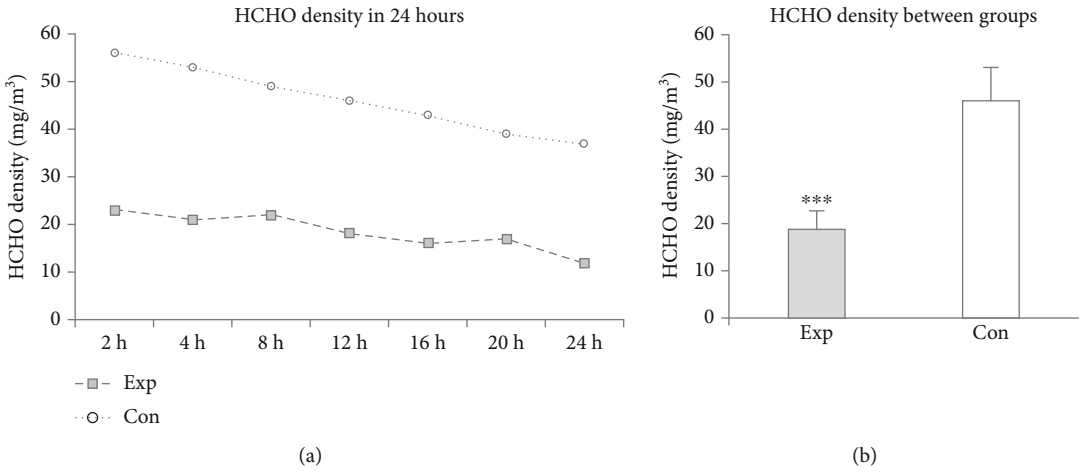

Figure 3: (a) The read of the HCHO tester 2 showed the condense of HCHO (mg/m^3) in the in every 2 hours after the valve was turned on for the experimental condition (in squares for ex1, sponge with bacteria R1) and the control (round circles for ck, empty sponge). The original condition was 60 mg/m^3 for each group, and the experimental group decreased significantly in the first test in the 2 h and after. The control group stably decreased from 60 mg/m^3 to about 40 mg/m^3 in 24 hours, and it might mean that the HCHO was continuously absorbed by the moisturized sponge or, maybe, diffused to the outer space of the box through the micro pores although the box was carefully sealed. (b) The removal efficiency in the process of the experiment slightly increased with time in the experimental condition.

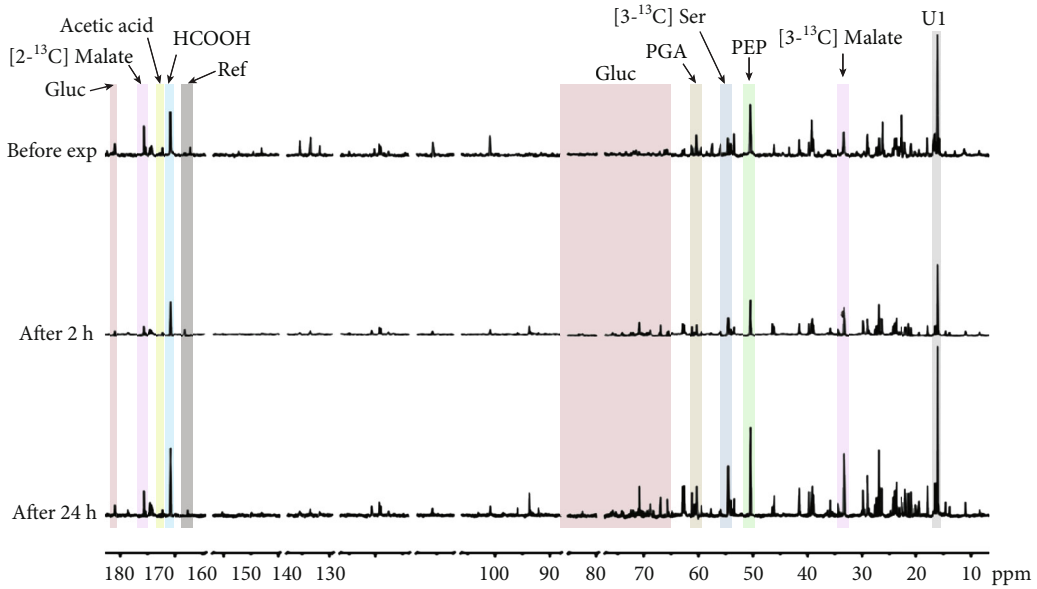

Figure 4: Signal intensity of different materials in ^{13}C-NMR before experiment (Before Exp), 2 hours after experiment start (After 2 h), and after 24 hours (After 24 h). Signal intensities for different materials were aligned vertically between different conditions. The height of vertical lines represents signal intensity of each material, and detailed intensities are listed in Table 1.

(Figure 5(a)). Group comparison between the different humidity levels shows significant differences between 18% and 30% and between 30% and 52%, respectively (Figure 5(b)). Results show that the 30% humidity of sponge of biofilter was the optimum among the three levels. In addition, in Figure 5, removal efficiency maintained relatively high and stable on 30% of humidity, while low and slightly increased on 18%, and between these two conditions, it slightly decreased on 52% during the time span of 60 minutes. This mean appropriate humidity optimized removal efficiency in the apparatus.

Then, when the humidity was fixed at 30% and the sponge layers was kept at 35 pieces in the second 3 sessions, the HCHO removal efficiency at 9.50 m^3/min of air flow was continuously higher than 4.75, 7.12 m^3/min levels across 60 minutes (Figure 6(a)). Significant differences between group comparisons show that the 9.50 m^3/min was the optimum (Figure 6(b)). In Figure 6, removal efficiency varied from different intensities of air flow, which was highest and very stable (around 90% during 60 minutes) under 9.5 m3/min, while lower but fluctuated (in the range of 40%-80%) at 7.12 m3/min and 4.75 m3/min. This is because the cylinder

Table 1: Signal intensity of different materials (*mean* ± *SD*) in ^{13}C-NMR in different time point before experiment (Before Exp), 2 hours after the experiment starts (After 2 h), and after 24 hours when the experiment was ended (After 24 h).

Materials	Before Exp	After 2 h	After 24 h
HCHO (HCOOH)	9.34 ± 0.01	5.5 ± 0.01	11.09 ± 0.02
Phosphoglycerate (PGA)	2.202 ± 0.005	1.29 ± 0.005	3.56 ± 0.005
Serine (Ser)	2.96 ± 0.01	3.16 ± 0.01	9.03 ± 0.01
Malic acid (Malate)	2.07 ± 0.01	5.31 ± 0.01	12.39 ± 0.01
Glycine (Gly)	3.66 ± 0.01	1.02 ± 0.01	6.01 ± 0.01
Glucose (Gluc)	36.44 ± 0.01	24.81 ± 0.01	48.07 ± 0.01
Phosphoenolpyruvate (PEP)	8.74 ± 0.01	6.16 ± 0.01	13.15 ± 0.01

Note: all group comparisons between each of the time point for each material were of significance ($P < 0.001$).

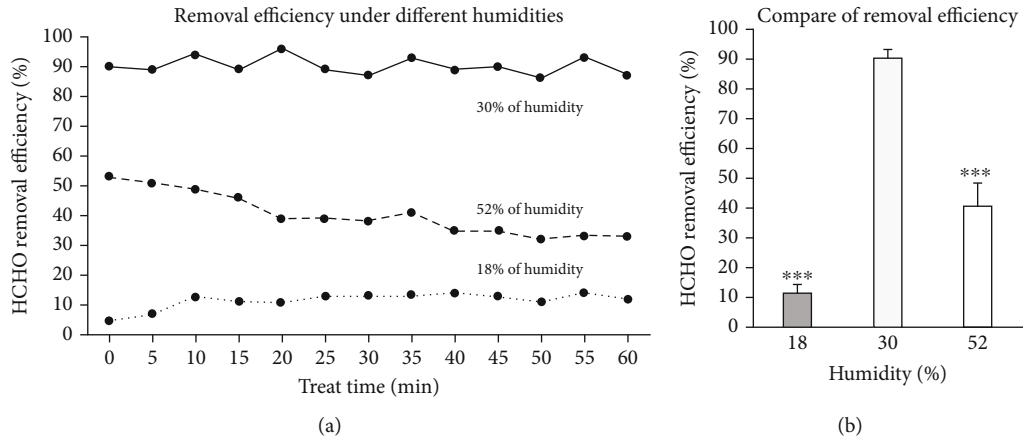

Figure 5: (a) Removal efficiency under different levels of humidity (18%, 30%, and 52%) in the sponge of biofilter with 35 layers and 9.50 m^3/min of air flow across 60 minutes sampled at every 5 minutes. (b) Group comparison of removal efficiency (*mean* ± *SD*) within different levels of humidity. *** Significant differences ($P < 0.001$) were found between 18% and 30% group (marked on 18% bar) and between 30% and 52% group (marked on 52% bar).

of the filter was filled with 35 layers of sponge with 30% of humidity, and there is a fluid retardation effect for air which flows through the sponge because of relatively high friction for air.

In the last 3 sessions, the sponge layers were of 15, 25, and 35 pieces while the humidity was kept at 30% and the air flow at 9.50 m^3/min. HCHO removal efficiency was continuously higher in 35 pieces group than the other two groups across 60 minutes (Figure 7(a)), and the group comparison shows that the 35 pieces group was the optimum (Figure 7(b)).

The results shows that to obtain the optimum effect of HCHO removal efficiency, the combination for the biofilter in current study was 35 pieces of sponge layers in cylinder to construct the core part of the biofilter, 30% of humidity, and 9.50 m^3/min of air flow by fans. With the combination of these optimum parameters, the removal efficiency can be as high as around 90% (Figure 8(a)) and elimination capability can be up to 24111-27000 μg/(m^3∗h) (Figure 8(b)) during 60 minutes of testing period.

3.3. Experiment 3: Biofilter with S-R1 Was Effective in a Room Treatment. Based on the results from experiment 2, an engineering applicable design as 35 layers of sponge in the cylinder with 30% of humidity and 9.50 m^3/min of air flow was used in this experiment in order to clear HCHO in a real polluted room. The initial HCHO density in the room was 1.60 mg/m^3 which was much higher than the official standard for safety (0.08 mg/m^3, GB/T 18883-2022). The read of the density continuously decreased across days, till the 19th day; the value decreased to 0.02 mg/m^3, which was below the standard value, and kept in 0.02-0.03 mg/m^3 range during the following days till the end of the experiment (Figure 9(a)). The mean values of density in the first 10 days (1-10 days) was significantly higher than the second 10 days (11-20 days, T-test, $P < 0.001$), as well as significantly higher than the third (21-30 days, T-test, $P < 0.001$) (Figure 9(b)).

The removal efficiency was optimum in the first day and as high as 93% with the biofilter was constructed under the optimized parameters obtained in experiment 2, while the HCHO removal efficiency declined from 93% to 54% during the initial period (1-5 d). But in the following days, it increased again and stabilized to around 80% in the last 10 days during the 30 days as the whole process (Figure 10(a)). The elimination capability also fluctuated during the 30 days, and the trend of change was very similar to removal efficiency (Figure 10(b)).

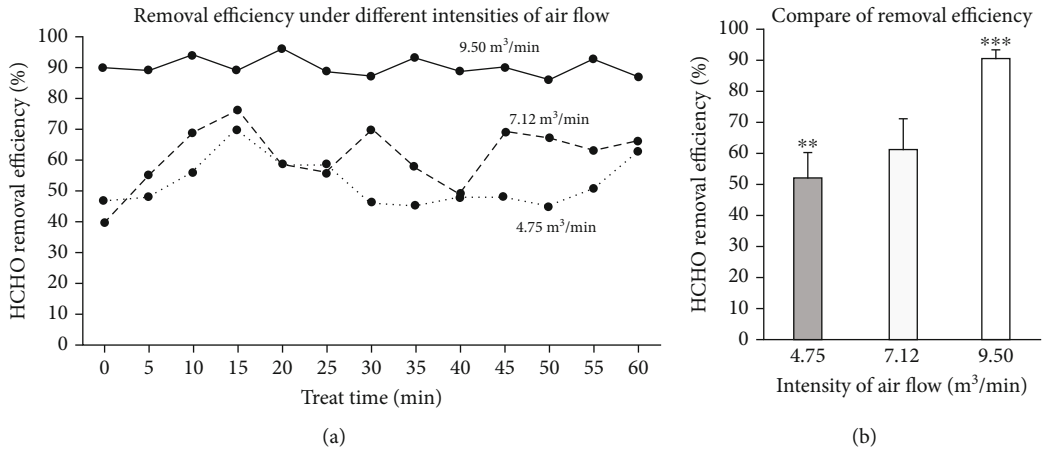

Figure 6: (a) Removal efficiency under different intensities of air flow (4.75, 7.12, and 9.50 m^3/min) with 35 layers and 30% of humidity in the biofilter across 60 minutes sampled at every 5 minutes. (b) Group comparison of removal efficiency (*mean* ± *SD*) within different levels of air flow. **Significant differences ($P < 0.01$) were found between 4.75 and 7.12 m^3/min group (marked on 4.75 bar). ***Significant differences ($P < 0.001$) between 7.12 and 9.50 m^3/min group (marked on 9.50 bar).

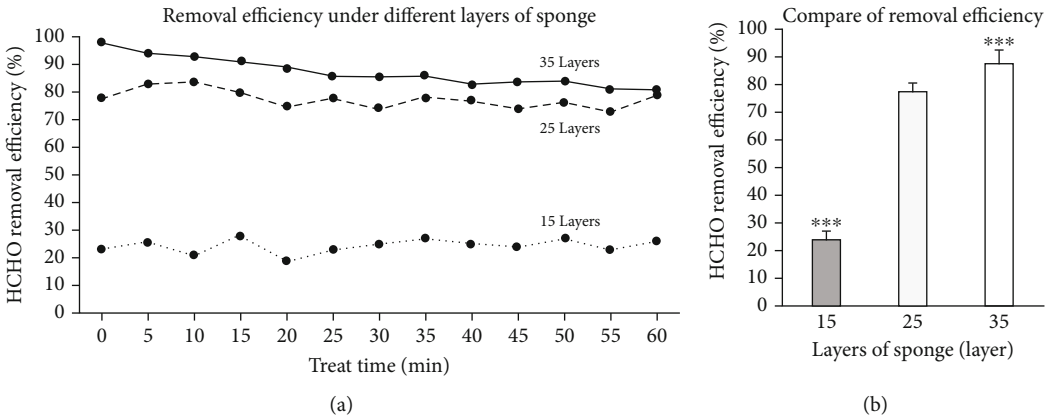

Figure 7: (a) Removal efficiency under different layers of sponge in the cylinder of biofilter (15, 25, and 35 layers) with 9.50 m^3/min of air flow and 30% of humidity in the biofilter across 60 minutes sampled at every 5 minutes. (b) Group comparison of removal efficiency (*mean* ± *SD*) within different layers of construction. ***Significant differences ($P < 0.001$) were found between 15 and 25 layers group (marked on 15 bar) and between 25 and 35 layers group (marked on 35 bar).

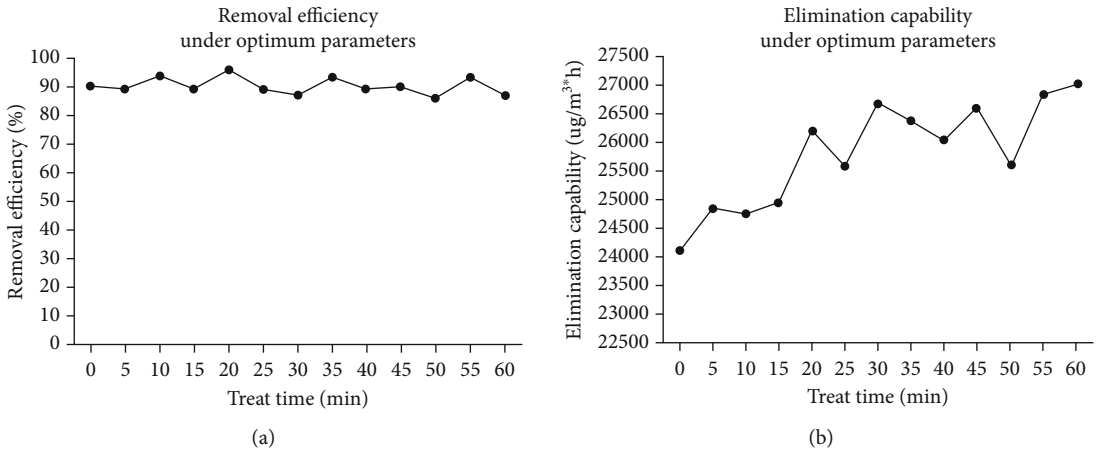

Figure 8: (a) Removal efficiency under optimum parameters of combination of 35 pieces of sponge in the cylinder, 30% of humidity, and 9.50 m^3/min of air flow across 60 minutes sampled at every 5 minutes. (b) Elimination capability under same combination of optimum parameters.

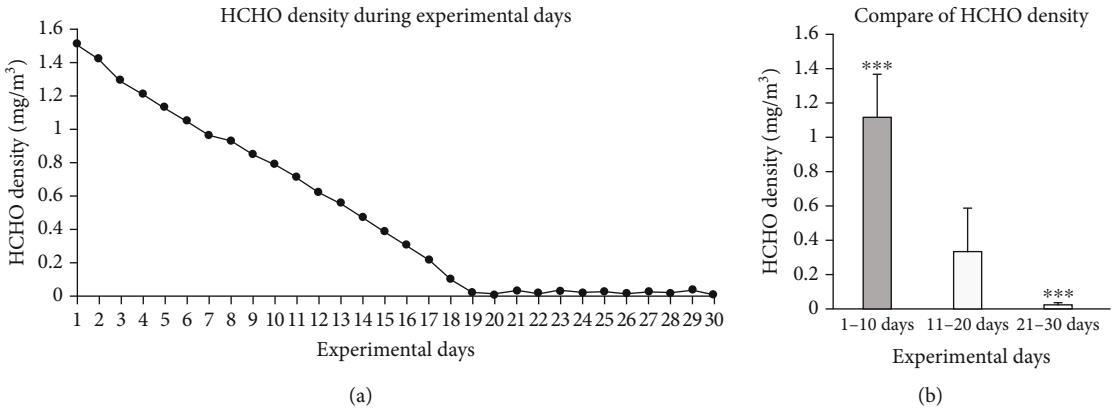

Figure 9: (a) HCHO density measured in the room in each day of the 30 days in experiment 3. The density continuously decreased from the original 1.6 mg/m^3 to 0.02 mg/m^3 on the 19th day and stabilized in later days, under the safe level of 0.08 mg/m^3 for indoor air (GB/T 18883-2022). (b) There were significant differences ($^{***}P < 0.001$) of the HCHO density between the first 10 days and the second 10 days (marked on 1-10 days bar), between the second 10 days, and the third 10 days (marked on 21-30 days bar).

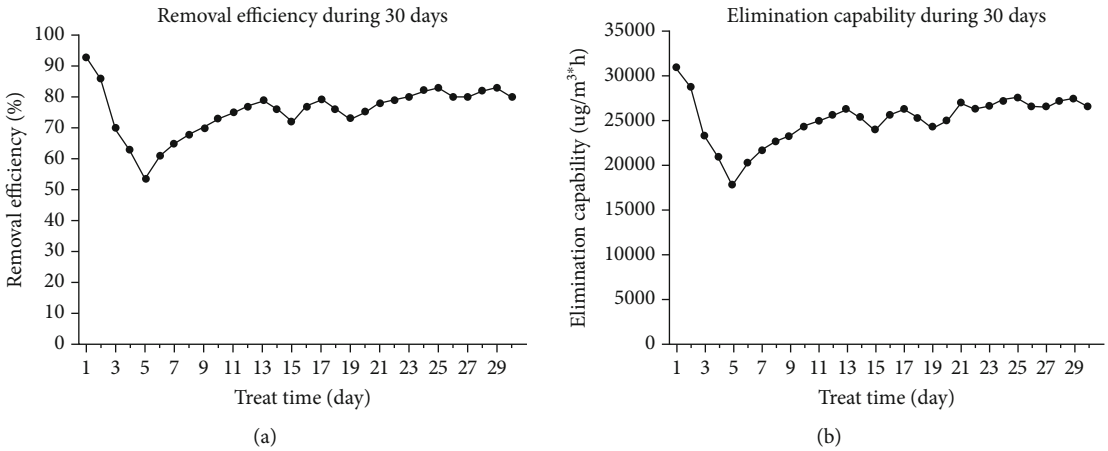

Figure 10: (a) Removal efficiency of the biofilter during 30 days in experiment 3. Constructed with optimized parameters obtained in experiment 2, the removal efficiency in the long run was originally as high as 93% in the first day, while it decreased to 54% from the second day to the fifth day and then continuously increased to around 80% in later days. (b) Elimination capability during 30 days with the trend of change similar to the removal efficiency.

The photographs of the packing material taken after 30 days of operation in the bioreactor showed that S-R1 still colonized the biofilter (Figure 2(d)). We collected inlet and outlet gas and treated LB agar plate with it. As shown in Figure 2(e), the gas at the outlet was relatively clean, and no microorganisms were detected after the plate was treated for 10 min.

4. Discussions

Formaldehyde (HCHO) belongs to group I carcinogen and can cause toxicant effect on the health of human when concentrated in an indoor environment. Chemical or physical methods are widely used to remove HCHO from an indoor environment, but some may bring side effects or second pollution. Current study suggested that a newly isolated bacterium S-R1 was effective in removing the HCHO in the air from a sealed room and is potentially developed for industrial use as a biofilter which has no side effect or potential secondary hazard.

To investigate the effectiveness and efficiency in removing HCHO in the air of an indoor environment, we designed three experiments with two different designed instruments as biofilters.

The purpose of experiment 1 was to test the effectiveness of S-R1 in removing HCHO from the air. To test this, a passive mode of biofilter constructed by a sponge layered on the bottom of the container was designed to prove whether S-R1 worked for a limited and small space containing HCHO. The results showed that the S-R1 was involved in degrading HCHO when they were embedded in the sponge which might have made contact with formaldehyde molecules in the air through molecular movement. This is the first time to prove that the S-R1 was effective in purifying HCHO in air, compared with that in previous research; this kind of bacteria was capable of degrading HCHO dissolved in water. The test of metabolic mechanism of H^{13}CHO in S-R1 cells

with ^{13}C-NMR analysis proved that this bacterium was actually involved in the biological HCHO-degrading process. Furthermore, the metabolite type and metabolic mechanism in S-R1 cells show some similarities to those of plant tissues such as gold poths [36], *Arabidopsis* [5], and banana leaves [7].

As the S-R1 bacteria were effective in removing HCHO in the air tested in experiment 1, we designed a prototype of active biofilter to force the air flow through the sponge filled with S-R1 for the next 2 experiments (Figure 2(a)). Previous studies had observed that the removal efficiency of a biofilter was correlated with the quantity of microorganism in the reactor [31, 37, 38]. However, it does not mean "more is better," because overstuffing the bed material of bacteria in the reaction chamber could barrier the contact between bacteria and polluted air, harm the mobility in the chamber, and decrease the air flow in the biofilter. These overall effects would result in a decrease of removal efficiency. Previous research also proposed that the air flow was one of the key parameters of a biofilter [23, 29, 39]. Except for the air flow, the humidity of the stuffed material in the reaction chamber is another key factor to influence removal efficiency. Low humidity would cause inactivation of the microorganism as well as the cross flow of the air in the overdried material and then decrease the removal efficiency. On the other side, overhumidity would decrease the area of liquid-air surface around the bacteria to form anaerobic zone and then cause anaerobic degradation in the microorganism to release odor and finally decrease removal efficiency [40–42]. In fact, previous researches found that problems that occurred in biofilters as high as 75% were caused by inappropriate controls of humidity [43, 44]. So the amount of bed material (as the number of bacteria), air flow, and humidity are the most important factors for the removal efficiency of pollution in the air of a biofilter.

In experiment 2, current study is an engineering-based research with the purpose of instrument development. So we focused on the three factors, humidity in the sponge, air flow, and layers of the sponge, which are key to the apparatus. We designed a prototype instrument to test the optimum combination of amount of imbed sponge, air flow, and humidity for a biofilter. This instrument was placed in a sealed empty room, and an HCHO evaporator was used to create 1.00 mg/m^3 of HCHO density in the air for experiment. We tested a total of 9 sessions to select the optimum parameters for a biofilter and found that 35 pieces of sponge, 30% humidity, and 9.50 m^3/min of air flow were the ideal combination to obtain best removal efficiency as high as around 90% (Figure 8(a)) and elimination capability can be up to 24111-27000 μg/(m^3∗h) (Figure 8(b)) during 60 minutes of testing period for the instrument used in current experiment. Compared to previous studies, such as the 70-95% of removal efficiency and 15-160 mg/(m3∗h) of elimination capability for removing formaldehyde in a biofilter by sodium alginate entrapment of pseudomonas putida [23] and 93-98% of removal efficiency by humidity from 15% to 30% for removing formaldehyde by using activated carbon, green apple roots, and activated sludge to form a biofilter, the efficiency in current study is comparable or even optimal than those studies. In the future research, we will continue other factors' investigations.

Experiment 3 lasted as long as 30 days and was a challenge to the stability of the instrument as well as the survival robustness of the bacteria. The results showed great effectivity and efficiency in removing HCHO in the air of this S-R1 bacterium. The biofilter took 19 days to totally clear the HCHO in the sealed room and maintain very low level of 0.02 mg/m^3 in the rest of the experimental days, compared to the standard safe level of 0.08 mg/m^3 for indoor air (GB/T 18883-2022). The instrument worked well during the whole process of the experiment, and the number of bacteria after 30 days of experiment was similar to that before experiment. This means that the bacterium was robust and can be alive in relatively long period under working condition. This characteristic of bacteria S-R1 makes advantage to develop engineering use biofilter in future. The photographs of the packing material taken after 30 days of operation in the bioreactor showed that S-R1 still colonized the biofilter. This means our biofilter is stable and sustainable. No bacterial leakage was found in the downstream gas detection as the research said the gel network formed by sodium alginate-calcium can integrate micrometer-scale cells into the network, while nanoscale molecules such as water and small molecular nutrients can free access the micropores [45].

In future study, on the basis of the previous studies [46, 47], we will look for substances similar to neutralizing metabolites to be added to the nutrient solution to increase the formaldehyde removal efficiency of the strain and understand its metabolic mechanism through molecular omics studies to provide a basis for subsequent genetic engineering operations.

In recent years, the continuous occurrence of air pollution incidents has greatly promoted the public's demand for air purifiers, and the market of biofilter for indoor air pollution has also gone up. The design of the biofilter provides a theoretical basis for the subsequent production of purifier products.

Data Availability

The data that support the findings of this study are available on request from the corresponding authors.

Conflicts of Interest

The authors declare no competing interests.

Authors' Contributions

Ru Wang was responsible for the investigation, analysis, and interpretation of results and wrote the original draft. Wenyuan Wang, Zhenyang An, and Zhengxue Li were responsible for the data collection, analysis, and interpretation of the results. Dongming Zhou and Wei Zhang were responsible for the statistics, composition of the figures and tables, drafting and writing, and review and editing. Yong Min was responsible for the methodology and writing—review and editing. Ru Wang, Wenyuan Wang, and Wei Zhang contributed equally to this work.

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