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Utility of Recycled Bedding for Laboratory Rodents

Toru Miyamoto,[1, 2, 3,*] Zhixia Li,[2] Tomomi Kibushi,[1] Shinya Okano,[1] Nakamichi Yamasaki,[2] and Noriyuki Kasai[1]

Animal facilities generate a large amount of used bedding containing excrement as medical waste. We developed a recycling system for used bedding that involves soft hydrothermal processing. In this study, we examined the effects of bedding type on growth, hematologic and serum biochemical values, and organ weights of female and male mice reared on either recycled or fresh bedding from 3 to 33 wk of age. Neither growth nor physiology differed between mice housed on recycled bedding compared with fresh bedding. When 14-wk-old mice were bred, litter size and total number of weaned pups showed no significant differences between animals raised on recycled or fresh bedding. Because bedding type influences the environment within cages and animal rooms, we evaluated particulate and ammonia data from cages and animal rooms. Values were significantly lower from cages and rooms that used recycled bedding than from those using fresh bedding, thus indicating that recycled bedding has the potential to improve the environment within both cages and animal rooms. Overall, this study revealed that recycled bedding is an excellent material for use in housing laboratory rodents. Specifically, recycled bedding may reduce medical waste and maintain healthy environments within cages and animal rooms.

Cage bedding is one of the most important environmental factors that can influence the wellbeing of laboratory rodents and, consequently, the experimental data obtained from those animals. In addition, bedding can give rise to environmental pollutants such as dust particulates, which affect air quality. Furthermore, ammonia levels vary with the ability of the bedding material to absorb ammonia. Air quality influences not only animal wellbeing but also the health of caretakers and scientists who engage in laboratory animal experiments.[3,9,16,22,26] Several studies have attempted to develop both bedding with desirable characteristics and the means of effectively evaluating those characteristics.[4,6,10,15,17,20,21] Although widely used in Japan, softwood shavings contain volatile and harmful components, including terpenes and aromatic compounds, and emit aromatic hydrocarbons that induce hepatic microsomal enzymes and cytotoxicity in animals.[7,9,11,18,19,23-25,27,28] In addition, a large amount of used bedding containing excrement and urine is discharged as industrial waste from life science institutes and breeding facilities. Used bedding is disposed of as hazardous waste, and this costly process is a burden on research expenses. Few studies[5] have attempted to improve bedding quality, but none has addressed recycling bedding materials.

We developed a system that uses high-temperature and -pressure dry steam in 'soft hydrothermal processing' to both improve fresh bedding and recycle used softwood bedding.[13] This system also removes both harmful organic components and aromatic hydrocarbons that could affect an animal's metabolism, such as by inducing cytochrome P450 in mouse liver.[12] Soft hydrothermal processing is a means of producing dry steam and treating biomass.[29] This extraction technique is based on the use of water as a solvent both at 100 to 200 °C and a pressure below saturated vapor pressure. This process lies in the low-density water molecular area of the steam field and is characterized by a lower dielectric constant (ε) than that of ordinary water. This process can accelerate the extraction of organic compounds.[1] In a previous study,[13] we reported that soft hydrothermal processing removed most of the predominant aromatic hydrocarbons from fresh bedding and the predominant harmful organic compounds derived from excrement and increased the adsorptive efficiency for ammonia gas. Therefore, the purpose of the present study was to evaluate the utility of recycled softwood bedding produced through soft hydrothermal processing for maintaining laboratory rodents.

Materials and Methods

Animals. To evaluate the effects of bedding type on growth, reproductive rates, hematologic values, serum biochemical values, and organ weights, Slc:ICR mice (Japan SLC, Hamamatsu, Japan) were kept in HEPA-filtered air-conditioned animal rooms at 12 to 15 air changes hourly, at 12:12-h light:dark cycle (lights on, 0800 to 2000), ambient temperature of 23 ± 3 °C, and relative humidity of 50% ± 10%. To evaluate the effect of recycled bedding on animal room environments, 2 animal rooms of similar type (nos. 625 and 627) containing several different mice strains (Table 1) each were provided with 3 sample cages containing male Slc:ICR mice (n = 3 or 4 per cage; age, 13 to 15 wk). Mice were kept in an SPF animal facility where quarterly surveillance confirmed that sentinel mice were negative for *Citrobacter rodentinm*, *Corynebacterium kutscheri*, *Mycoplasma pulmonis*, *Pasteurella pneumotropica*, *Salmonella* spp., *Clostridium piliforme*, *Ectromelia virus*, lymphocytic choriomeningitis virus, mouse hepatitis virus, Sendai virus, ectoparasites, intestinal protozoa, and pinworms. Polyolefin cages (225 × 338 × 140 mm, Clean S, CLEA Japan, Tokyo, Japan) were changed once weekly at the same time as the bedding changes. Autoclaved standard rodent chow (Labo MR, Nosan, Yokohama, Japan) and tap water via drinking valve were available ad libitum. Animal experiments were conducted under strict ethical considerations for the use of laboratory animals, according to the procedures outlined by

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[1]Institute for Animal Experimentation, Graduate School of Medicine and [2]Graduate School of Environmental Studies, Tohoku University, Sendai, Japan; [3]Maeda Seisakusho Company, Nagano, Japan.
**Corresponding author. Email: miyamoto@mail.tains.tohoku.ac.jp*

Table 1. Number of cages and mice in the animal room in which airborne dust particles content and ammonia concentration were measured

	Animal room	Type of bedding	No. of cages[a]	No. of mice[b]	Weight of mice (g)[c]
First term (29 July–8 September)	625	Fresh bedding	152.4 ± 5.5	364.2 ± 28.0	47.18 ± 4.34 (n = 11)
	627	Recycled bedding	110.8 ± 3.4	400.6 ± 13.4	43.83 ± 4.77 (n = 12)
Second term (9 September–21 October)	625	Recycled bedding	147.7 ± 6.0	348.3 ± 24.9	49.98 ± 4.21 (n = 12)
	627	Fresh bedding	114.8 ± 5.0	379.3 ± 13.9	44.24 ± 4.54 (n = 12)

Results are expressed as mean ± 1 SD
[a]The daily average number of cages in the 4 racks in the room.
[b]The daily average number of mice in the 4 racks in the room.
[c]The daily average body weight of the mice in the 3 sample cages.

the *Guidelines for the Care and Use of Laboratory Animals of Tohoku University*. All studies were preapproved by the Animal Care and Use Committee of Tohoku University Graduate School of Medicine.

Bedding. Two types of bedding, fresh and recycled, were used. The fresh bedding was made from softwood spruce (*Picea sitchensis*), shaved to rectangular chips (Tokoziki, Dohoh-rika, Sapporo, Japan) of approximately 10 × 15 × 0.3 mm. Dirty bedding was recycled through soft hydrothermal processing. No additional nesting or other enrichment materials were used because of the complication of removing their fragments from treated bedding. Bedding was processed in a large-scale apparatus (prototype model, Maeda Seisakusho, Nagano, Japan) as previously described.[13] Briefly, dry steam maintained at 150 °C and 0.45 MPa for 90 min was allowed to flow forward through a cylindrical reactor with a 158.3-L volume. The cylindrical reactor was filled with dirty bedding for drying. The pressure of the dry steam was adjusted by controlling the back-pressure regulator, and the mixing ratio of vapor and nitrogen gas was adjusted by using the mass flow controller (Figure 1). Dry steam was carried into the reactor and allowed to flow forward through the sample holders for extraction of volatile compounds and other lipophilic substances. The dry steam then flowed into the condenser, where the exhausted steam was condensed below 20 °C. Afterward the condensed extract compounds were fed directly into the reservoir, and separated into gas and liquid phases for further analysis. Finally, the gas phase was deodorized and released outside.[13] After treatment, the bedding was shaken for 1 min in a portable sieve shaker with a 5-mm Japanese Industrial Standard sieve to remove debris, residual excrement, and dust particles in the recycled bedding. Because the bedding particles were larger than the fecal pellets, the sieve completely removed the excrement and dust particles. The recycled bedding was sealed in plastic bags until use. Both types of bedding were autoclaved (121 °C, 20 min) before use.

Growth and physiology of mice reared on each type of bedding. Mice (20 male; 20 female; age, 3 wk) were marked for identification, weighed, and randomly allocated by sex and weight into a fresh bedding group and a recycled bedding group. Each cage contained 5 mice and was supplied with approximately 50 g of either fresh or recycled bedding. After 1 wk of acclimation, individual body weights were recorded weekly from 4 to 33 wk of age. At 33 wk of age, after 16 h of fasting, animals were anesthetized with pentobarbital, and blood samples were collected by cardiocentesis into sterilized tubes containing dipotassium EDTA or clot tubes. Animals

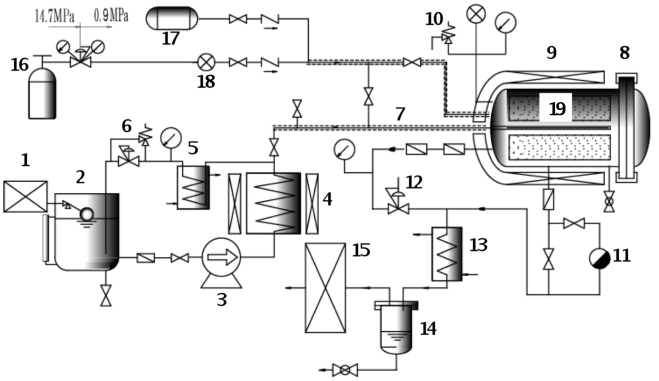

Figure 1. Schematic diagram showing the flow-type extraction apparatus used during soft hydrothermal processing. 1, water softener; 2, water tank; 3, plunger pump; 4, steam generator; 5, condenser; 6, back pressure regulator; 7, line heater; 8, reactor; 9, reactor heater; 10, safety valve; 11, steam trap; 12, control valve; 13, condenser; 14, extract reservoir; 15, deodorization; 16, N_2 gas; 17, compressor; 18, N_2 gas mass flow controller; 19, sample holders.

then were euthanized by cervical dislocation and organs excised. Blood samples were evaluated for hematologic parameters including hemoglobin, RBC count, WBC count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean cell hemoglobin concentration, total bilirubin, triglycerides, total cholesterol, glucose, aspartate aminotransferase, and alanine aminotransferase. Excised organs (brain, heart, kidney, liver, lung, spleen, adrenal, testis, and uterus) were weighed.

Reproductive rates of mice housed on each type of bedding. At 14 wk of age, 20 male and 20 female Slc:ICR mice (14 wk of age) that were reared as described earlier were bred by placing 1 mouse of each sex into each cage and monitoring once daily for vaginal plugs. Once vaginal plug was detected, the male mouse was removed from the cage, and the pregnant female mouse was individually housed until birth with either type of bedding. Newborn pups were weaned at 3 wk of age and litter size and the number of weaned pups were recorded.

Airborne dust and ammonia content in animal rooms. All mice were maintained in cages that contained approximately 50 g of either type of bedding in 2 animal rooms (rooms 625 and 627). Both rooms were the same size [2900 (width) × 5400 (length) × 2400 (height) mm] and were air-conditioned at a air exchange rate of 12 to 15 times hourly. Each room contained 4 ventilated mouse cage racks (AR-18SM, Toyoriko, Tokyo, Japan) with

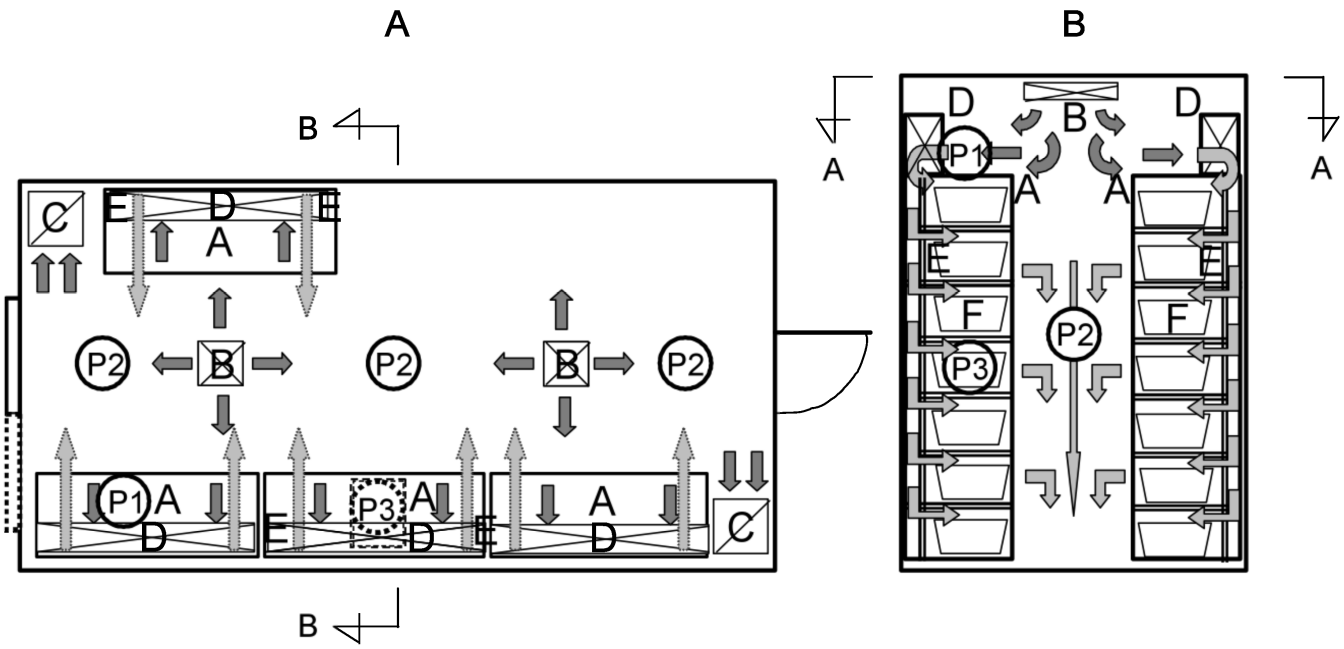

Figure 2. Schematic diagram of the ventilation system and airflow in animal rooms. Both animal rooms were the same size [2900 (width) × 5400 (length) × 2400 (height) mm] and were air-conditioned with 12–15 fresh air exchanges hourly. A, Mouse racks with positive-pressure laminar-flow hood ventilation systems with high-efficiency fibrous filters; B, supply air inlet on the ceiling; C, exhaust air outlet from the room; D, air inlet to the mouse rack through high-efficiency fibrous filters; E, high-efficiency fibrous filtered air supplied from slit-type diffusers; F, cage; P1, measurement points for airborne dust particles; P2, measurement points for ammonia concentration; P3, measurement points for airborne dust particles and ammonia concentrations. Panel A is an overhead view of the animal room in the direction of the arrows labeled A in Panel B, a side view of the animal room in the direction of the arrows labeled B in panel A.

positive-pressure laminar hoods equipped with high-efficiency fibrous filters (TAR-18SM, Toyoriko; Figure 2). Each rack was 7 shelves tall by 6 cages wide, providing a room capacity of 168 cages. Each rack was provided with 3 sample cages each containing 3 or 4 mice. High-efficiency fibrous filtered air was supplied by slit-type diffusers from back of the rack system through cages to the room. These high-efficiency fibrous filters are similar to HEPA filters, but their filtering performance is not defined as strictly in the Japanese Industrial Standard as that of HEPA filters. Our preliminary measurements of airborne dust particles per cubic foot yielded 0 particles of any size at the slit-type diffusers.

About 350 to 400 mice of several different strains were maintained in 110 to 150 cages with a single type of bedding in each room for 42 d (first term). Cages were changed every 7 d. To avoid biased data due to differences between the animal rooms, the bedding in each room was exchanged to the other type and maintained for an additional 43 d (second term). Effects of bedding on the animal room environments were compared between the first and second term in each room to avoid an additional confounder. Ammonia concentration in the animal room and sample cages was measured immediately before and after changing the cages and on the fourth day after cage change for 6 wk. The amount of airborne dust particles in sample cages was recorded 5 times consecutively before and immediately after changing the cages and on the fourth day after changing cages for 3 wk. The airborne dust particle content in the animal room was recorded continuously every 20 min for 7 d. The number of airborne dust particles was measured by using a handheld particle counter and laser diode sideways light-scattering methods (model KR-12A, Rion, Kokubunji, Japan). The counter automatically simultaneously measured airborne dust particles that were 0.3, 0.5, 0.7, 1.0, 2.0, and 5.0 µm in size. Particulates at the entrance to the high-efficiency fibrous filters (the room location yielding the highest counts) and in the 3 sample cages placed in the central row of the middle shelf (Figure 2) were measured. Ammonia concentrations were determined by using a gas-sampling pump kit (model GV-100S, Gastec, Ayase, Japan) and quick-measuring detector tubes (Gastec tube Number 3L) with a detection range of 2 to 30 ppm. Single pulling bellows pump-strokes (100 mL; as specified for the measurement of ammonia by the manufacturer) were performed with the detector tubes placed at a height of 1 m from the floor in the center and at both sides of the animal rooms (Figure 2, P2) and at the center of the cage at 10 cm from the bottom.

Statistics. All results were expressed as mean ± 1 SD. Differences were analyzed by using Student *t* tests, Tukey's honestly significant difference adjustments, and 2-way ANOVA. Statistical significance was preset at a *P* value of less than 0.05. Analyses were performed by using the Excel spreadsheet program (Office 2003, Microsoft, Redmond, WA).

Results

Influence of recycled bedding on the growth and physiology of mice. Male mice housed on either fresh or recycled bedding showed normal increases in body weight, and no differences between groups were apparent after 33 wk of rearing (99.6% power at the 5% level of significance). After 9 wk of age, female mice housed on recycled bedding showed significant ($P < 0.05$) increases in body weight as compared with those housed on fresh bedding (Figure 3 A). No significant differences in organ weights (Figure 3 B) or hematologic and serum biochemical values (Table 2) were seen between male and female mice maintained on fresh bedding or recycled bedding. These data revealed no negative effects of recycled bedding on growth, organ weights, or hematologic and serum biochemical values when compared with animals housed on fresh bedding.

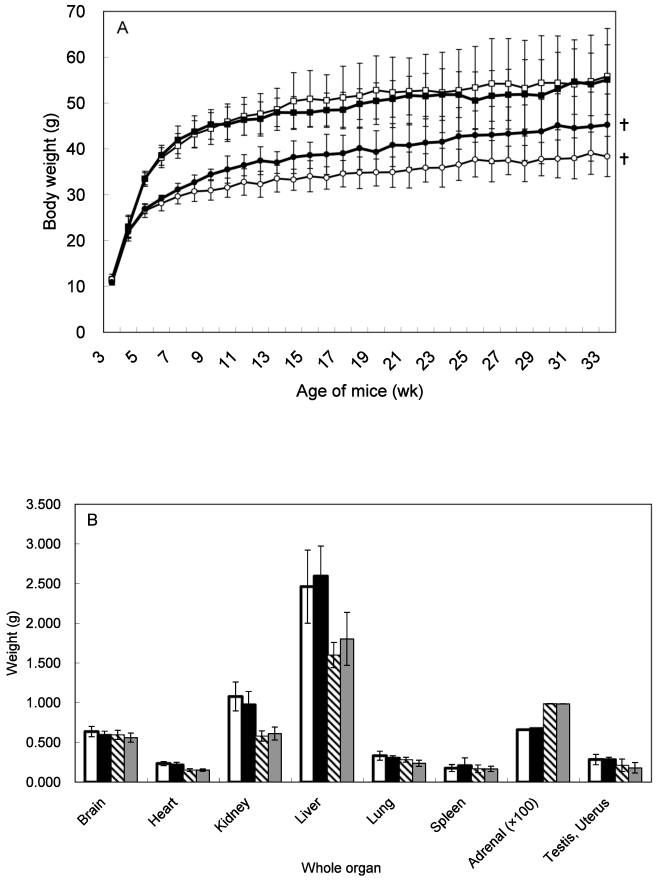

Figure 3. Growth curve and physiologic measurements of mice housed on fresh or recycled bedding. (A) Growth curve of ICR mice housed on recycled or fresh bedding. Open squares with thin line, male mice housed on fresh bedding (n = 10); solid squares with thick line, male mice housed on recycled bedding (n = 11); open circles with thin line, female mice housed on fresh bedding (n = 10); solid circles with thick line, female mice housed on recycled bedding (n = 11). (B) Organ weights of ICR mice housed on either fresh or recycled bedding. Open bars, male mice housed on fresh bedding (n = 9); solid bars, male mice housed on recycled bedding (n = 9); hatched bars, female mice housed on fresh bedding (n = 11); shaded bars, female mice housed on recycled bedding (n = 10). Data are expressed as mean ± 1 SD. Significant (†, $P < 0.05$) individual differences were evaluated by using 2-way ANOVA.

Influence of recycled bedding on the reproductive rates of mice. To determine the influence of recycled bedding on reproductive rates, we recorded litter size and the number of pups weaned. The litter sizes of ICR mice housed on fresh bedding and recycled bedding were 12.8 ± 2.5 (n = 10) and 13.3 ± 3.6 (n = 10), respectively. The total number of pups weaned from parents housed on fresh bedding and recycled bedding was 8.5 ± 4.7 per dam (n = 10) and 8.8 ± 5.1 per dam (n = 10), respectively. No significant differences in litter size (93.5% power at the 5% level of significance) or the number of pups weaned (96.4% power at the 5% level of significance) occurred between groups. Overall, these results indicate no negative effects of recycled bedding (via soft-hydrothermal processing) on mouse reproductive rates.

Effects of recycled bedding on animal room environments. We examined the effects of recycled bedding on cage and animal room environments with regard to the levels of airborne dust particles and ammonia in the air. The concentration of airborne dust particles (diameter, 0.5 µm) in cages with recycled bedding was lower than that of fresh bedding at all time points ($P < 0.05$, Figure 4 A). The peak in particulates in cages with fresh bedding on day 4 of the first week was due to increased activity of the mice, which may have released fine fibers into the air. The average of all time points was significantly ($P < 0.05$) lower for recycled bedding (5416.2 ± 467.1 particles/ft^3) than fresh bedding (14795.3 ± 3759.4 particles/ft^3). Similar results were obtained for airborne dust particles 0.3, 0.7, 1.0, 2.0, and 5.0 µm in size (data not shown). In addition, the concentrations of airborne dust particles 0.5, 1.0, and 5.0 µm in size within animal rooms were similar to each other. During both day and night, significantly $P < 0.05$) lower levels of airborne dust particles were detected in rooms with recycled bedding as compared with rooms using fresh bedding (excluding particles 5.0 µm in size; Figure 4 B).

At 3 d after cage change, the ammonia concentration was significantly ($P < 0.05$) lower in cages with recycled bedding than in cages with fresh bedding (Figure 5 A). The results for day 1 were similar for fresh and recycled bedding, and a trend similar to that on day 3 occurred at 7 d after bedding changes (Figure 5 A). The average of these time points was also lower ($P < 0.05$) for recycled bedding (11.6 ± 10.3 ppm) than fresh bedding (14.3 ± 10.2 ppm). Similarly, ammonia concentrations in animal rooms with recycled bedding were significantly ($P < 0.05$) lower than those of rooms with fresh bedding (Figure 5 B). Overall, these results indicate that animal room environments improved with regard to the reduction of airborne dust particles and ammonia concentrations when recycled bedding was used.

Discussion

In this study, we found that recycled bedding (that is, used bedding treated by using soft hydrothermal processing) had no negative influences on the growth and reproductive rate of experimental mice. In addition, recycled bedding has the potential to improve cage and animal room environments over conditions associated with fresh bedding.

Softwood bedding is used commonly in animal facilities in Japan, but it emits aromatic hydrocarbons that induce hepatic microsomal enzymes and cytotoxicity.[7,9,11,18,19,23-25,27,28] We demonstrated previously that extraction and drying through soft hydrothermal processing reduces the concentration of aromatic hydrocarbons in softwood bedding as well as the most predominant harmful organic compounds derived from excrement in used bedding.[14] In contrast, common heat treatments such as autoclave sterilization use saturated steam, not dry steam. Saturated steam cannot effectively extract harmful organic components from used bedding and cannot dry bedding simultaneously during the sterilization process. We also demonstrated that the removal of these volatile compounds by soft hydrothermal processing decreases the hepatic P450 enzyme-inducing effects of red cedar (softwood) bedding.[13] Therefore, we examined whether recycled bedding has any negative effects on the growth, reproduction, or living environments of mice. Our data revealed no negative effects of recycled bedding on physiologic measurements (body weight, hematologic values, serum biochemical values, and organ weights) in mice reared for 33 wk with recycled bedding as compared with fresh bedding. No significant differences in litter size or the total number of pups weaned (that is, indexes of reproduction ability) between fresh bedding and recycled bedding occurred. In addition, concentrations of airborne dust and ammonia were decreased significantly in the air of cages and animal rooms using recycled bedding compared with those with fresh bedding.

Table 2. Hematologic and serum biochemical values of ICR mice on fresh and recycled bedding

	Male mice		Female mice	
	Fresh bedding	Recycled bedding	Fresh bedding	Recycled bedding
	$n = 4$	$n = 2$	$n = 5$	$n = 5$
Hemoglobin (g/dL)	13.10 ± 0.70	11.10 ± 0.99	12.98 ± 0.96	13.34 ± 0.43
RBC (×10000/mm^3)	810.50 ±32.15	631.00 ± 79.2	805.00 ± 69.24	824.60 ±21.24
WBC (/μL)	2625.00 ± 263.00	2550.00 ± 2474.87	600.00 ± 122.47	1160.00 ±798.75
Hematocrit (%)	46.55 ± 2.59	37.50 ± 3.25	43.92 ± 3.14	45.44 ± 1.97
Mean corpuscular volume (μm^3)	57.25 ± 0.96	59.50 ± 2.12	54.60 ± 2.41	55.20 ± 1.64
Mean corpuscular hemoglobin (pg)	16.00 ±0.00	17.50 ± 0.71	16.20 ± 0.84	16.40 ± 0.55
Mean corpuscular hemoglobin concentration (%)	28.00 ± 0.00	30.00 ± 0.00	29.60 ± 0.89	29.40 ± 1.52
	$n = 4$	$n = 4$	$n = 6$	$n = 5$
Total bilirubin (mg/dL)	0.07 ± 0.02	0.06 ± 0.02	0.047 ± 0.01	0.05 ± 0.01
Triglyceride (mg/dL)	61.50 ± 23.98	50.25 ± 16.01	36.67 ± 11.91	47.60 ± 17.18
Total cholesterol (mg/dL)	112.00 ± 7.12	148.00 ± 48.39	55.17 ± 12.45	70.00 ± 18.28
Glucose (mg/dL)	145.00 ± 61.93	128.00 ± 22.65	101.09 ± 21.40	115.70 ± 28.72
Aspartate aminotransferase (IU/L)	94.25 ± 26.11	164.75 ± 64.63	140.67 ± 27.80	131.00 ± 22.75
Alanine aminotransferase (IU/L)	27.50 ± 10.97	39.50 ± 13.80	32.00 ± 8.90	41.61 ± 6.35

Results are expressed as mean ± 1 SD

The principal mechanisms regulating the observed decreases in ammonia concentrations within cages and animal rooms involve carbonification of cellulose or lignin, the main components of wood, which actively occurs when temperatures reach 300 °C. As a result, acidic functional groups such as carboxyl and phenolic hydroxyl groups form on the surface of the wood.[2,15] We believe that the formation of acidic functional groups was initiated on the wood surface during soft hydrothermal processing by the promotion of dehydration below 200 °C. In addition, the softwood bedding obtained a high adsorptive potential for bases such as ammonia gas. Indeed, we demonstrated that the adsorptive capacity for gaseous ammonia of softwood bedding treated through the soft hydrothermal process was much greater than that of fresh bedding.[14]

Used bedding is disposed of as a hazardous waste. This process is costly and poses a high burden on research expenses. As we show here, however, soft hydrothermal processing is 1 method that effectively rids used bedding of many harmful compounds and makes it possible to recycle used bedding so that hazardous waste and associated expenses decrease. Soft hydrothermal processing also generates several additional positive characteristics in recycled bedding, such as high ammonia absorptive ability and reduction in airborne dust, as compared with fresh softwood bedding. Furthermore, 85% of used bedding can be recovered after 1 cycle of soft hydrothermal processing (15% of used bedding is discarded as residual material). Thus, used bedding disposal could be reduced by 30% with only 1 incidence of reuse. Multiple recycling of used bedding seems feasible but requires investigation, including of the associated potential degradation of bedding material. In addition, other types of bedding, such as wood-pulp–based contact bedding, may be amenable to recycling through soft hydrothermal processing.

In Japan, a kilogram of any type of fresh bedding costs approximately USD$3–4, and the expense of waste disposal (per kilogram) is approximately USD$0.20 (domestic waste) to USD$4–10 (industrial waste). The quantity of used bedding is 1.9 times that of fresh bedding, due to the presence of excrement in the used bedding. Therefore, a facility that consumes 20,000 kg fresh bedding annually experiences USD$67,600 in bedding-associated costs each year. In comparison, the approximate initial investment for the flow-type extraction apparatus used for soft hydrothermal processing (processing capacity, 160 kg daily) is USD$300,000, and the cost to perform the recycling is estimated at USD$4000 annually. Therefore, the initial outlay will be recouped within a few years. In addition, soft hydrothermal processing uses only water and does not require any harmful materials, such as organic solvent, and the mechanism can readily be scaled up to, for example, a facility with 20,000 cages. This cost analysis suggests that recycled bedding saves money and wood resources and mitigates greenhouse gas emissions from animal facilities.

Overall, the use of recycled bedding decreases hazardous waste and waste expenses. In addition, it improves the environment within cages and animal rooms. Our findings indicate that recycled bedding produced through soft hydrothermal processes is an excellent material for maintaining laboratory rodents within animal facilities.

Acknowledgments

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The soft hydrothermal processor was provided as a prototype model by Maeda Seisakusho.

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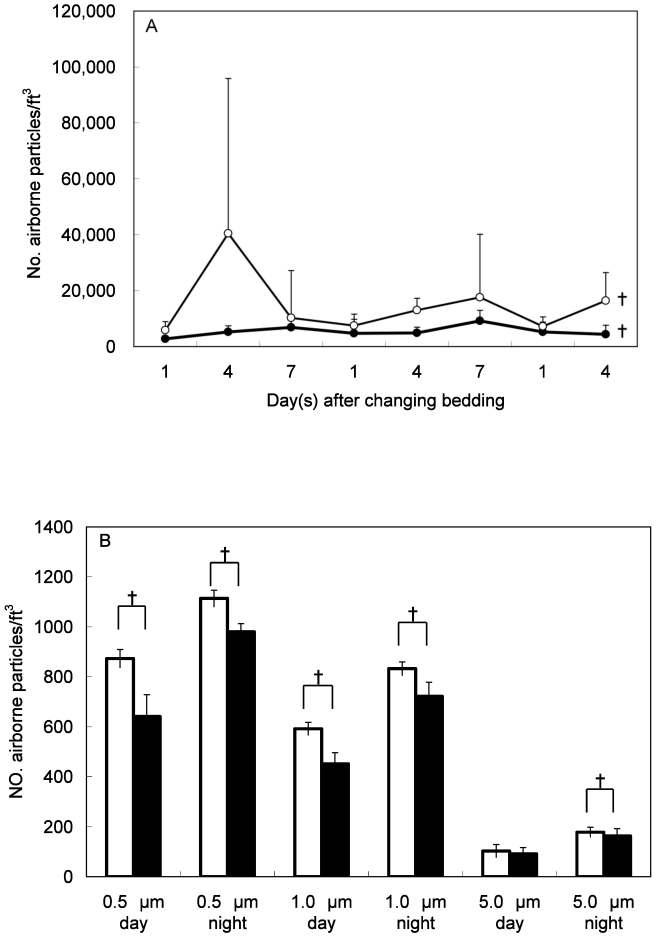

Figure 4. Airborne dust particles in cages and animal rooms using recycled bedding. (A) Airborne particles (0.5 µm) in sample cages were quantified at 1, 4, and 7 d after bedding changes and repeated 2 times (for a total of 3 wk). Open circles, cages with fresh bedding (n = 15); closed circles, cages with recycled bedding (n = 15). (B) Airborne dust particles (0.5, 1.0, and 5.0 µm) during the day or night in animal rooms. Open bars, animal room with fresh bedding (n = 252 at day, n = 288 at night); closed bars, animal room with recycled bedding (n = 216 at day, n = 252 at night). Data are expressed as mean ± 1 SD. Significant individual differences were evaluated by using a Student t test if the 2-way ANOVA was significant (†, $P < 0.05$).

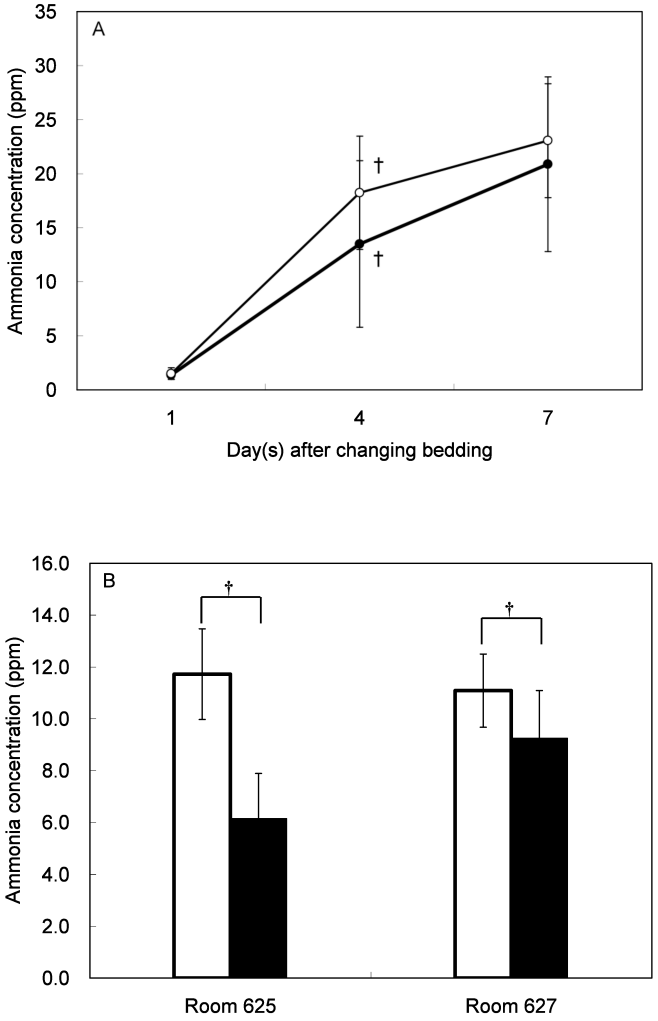

Figure 5. Ammonia concentration in cages and animal rooms using recycled bedding. (A) Ammonia concentration in sample cages. Open circles, cages with fresh bedding (n = 99); closed circles, cages with recycled bedding (n = 108). (B) Ammonia concentration in animal rooms. Open bars, animal room with fresh bedding (n = 45); closed bars, animal room with recycled bedding (n = 45). Data are expressed as mean ± 1 SD. Significant individual differences were evaluated by using a Student t test if the 2-way ANOVA was significant (†, ‡ shows $P < 0.05$).

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